Long Term Residual Effects of Lead Mining on Man and Grazing Livestock within a Rural Community in Southern Scotland.

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DECLARATION

I declare that this thesis was composed by myself and has not been accepted in any previous application for a degree. The work it describes and the ideas it contains are my own unless otherwise stated. Assistance given by other people has been acknowledged.

Wendy E. Moffat

ABSTRACT

The residual lead contamination of human and livestock populations was studied in two villages set in the Southern Uplands of Scotland, where the environment is heavily contaminated through former mining activity. A comparison was made of blood lead concentrations of representative samples of adult males (n=55; BPb:15.95 +/- 5.4 $\mu g100ml^{-1}$; $p<0.001$) and females (n=71; BPb:12.43 +/- 5.2 $\mu g100ml^{-1}$; $p<0.001$), and of all children (n=22; BPb:17.61 +/- 5.4 $\mu g100ml^{-1}$; $p<0.001$) living in the area with residents of an uncontaminated village. Possible routes of lead exposure involving domestic water, house dust, airborne dust, hands, food preparation surfaces, garden soils and home grown vegetable consumption were investigated. There was a general increase in lead in the environment in the contaminated villages and blood lead levels were between 45 and 70 per cent higher than the control village. The major determinants of blood lead in both areas were sought through correlation and multiple regression analysis. Lead in drinking water had the largest influence in explaining blood lead variability (11%), although levels were low and within EEC guidelines; hand lead accounted for 6%, airborne dust lead 3% and kitchen surface and house dust lead less than 1% of variation in blood lead. A parallel study was made of lead contamination of grazing lambs which can die as a result of a locomotor disorder in the same contaminated area. Representative samples were collected from both a contaminated and a control flock of ewes and lambs, for the measurement of both blood and milk lead in ewes and sequential blood concentrations in lambs. Other heavy metals (copper and zinc) and macronutrients (phosphorus and calcium) were additionally monitored in blood : mineral composition of lamb tissues, soil and herbage were also investigated. Blood lead concentrations in lambs from the contaminated flock reached a peak value of 1.45 +/- 0.89 μgml^{-1} shortly before the disorder habitually appears, strengthening the evidence for an association between lead exposure and death rates. Tissue lead concentrations in dead lambs were between six and fifty times higher than those in the control flock. Milk lead mean concentrations were 0.14 and 0.03 μgml^{-1}, respectively, in the contaminated and control flocks indicating mammary transfer of lead to be an important pathway of contamination. Low soil and pasture concentrations of phosphorus and calcium were established : young lambs fed supplements of these elements showed good locomotor ability and lower blood lead concentrations than unsupplemented lambs, but ascorbic acid supplements were ineffective. A final study in the former lead mining area attempted to reduce environmental lead exposure at source by establishing vegetation on the fine-grained dry residues from the old tailing lagoons which were subject to dispersal by wind and water erosion. Restoration techniques included trial plots amended with locally available soil, domestic refuse and colliery spoil at depths of between 150 and 450mm, and direct hydroseeding trials using both 'metal tolerant' and conventional seed mixes. Sewage sludge applications were made to increase soil fertility in the hydroseeding trials. A successful grass sward cover was achieved using both techniques. When sheep were grazed on restored spoil they showed only moderate increases in blood lead. The need for further restoration work is indicated to support the educational programme instituted in an attempt to reduce the lead contamination which has persisted since closure of the mines in the 1930s.

DEDICATION

To

IAIN

and MY FAMILY

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Its very difficult to know where to begin. Overall I seem to have involved, or indeed in some cases roped in, an enormous number of individuals in an effort to fulfil the aims of this research.

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A published paper entitled 'Blood lead determinants of a population living in a former lead mining area in Southern Scotland' is included as an appendix. Permission has been obtained from 'Environmental Geochemistry and Health' Publishers.

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THE UNIVERSITY *of* EDINBURGH

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1. INTRODUCTION : THE RESEARCH CONTEXT

1.1 GENERAL HISTORICAL PERSPECTIVE

Lead is the most abundant, and perhaps the most well known of the natural heavy metals and is normally found at concentrations of around 15 $\mu g g^{-1}$ in the earth's crust. Formed around one billion years ago in the earth's history lead ores are found primarily as insoluble lead sulphide or galena, with secondary forms of plattnerite (PbO_2), cerussite ($PbCO_3$) and anglesite ($PbSO_4$) less commonly found (Zimdahl and Hassett, 1979). As a result, lead is found world-wide in rocks, soils, air and waters in trace amounts from natural geological sources.

This primarily low level input to the environment is however, of little significance compared to the metal's distribution as a result of man's activities which have resulted in lead becoming one of the most widely dispersed environmental contaminants on a global scale. Lead deposits sufficiently rich to justify mining have been found localised and widely dispersed in all five continents of the world (Lead Development Association, 1974), the physical and chemical characteristics of the metal, including its general ease of use and attainable melting point of 327°C leading to its early use by man (Davies, 1987).

It is known that small amounts of lead were used by Egyptians, Phoenicians and Hebrews for making glazes, glass and pigments over 1000 years B.C. By Roman times, large quantities of lead were employed for architectural purposes including the supply of plumbing, and also for use in roofing materials, drinking vessels, cooking pots and pigments. With the fall of Rome came a resultant decline in the demand for lead and it was not until the Middle Ages that it was once again mined in Great Britain

and used for purposes of roofing, piping and warfare. However it was the onset of the Industrial Revolution which saw a marked widespread increase in lead mining and production. Many of the modern uses for lead, for example the lead storage battery and the use of lead as electrodes in accumulators for the storage of electricity, were invented in this period (Lansdown and Yule, 1981).

Today lead is present in considerable quantities in the modern environment not only from mining and smelting of ores, but also from its use in industrial and urban processes; as a polymerisation agent in paints; as an additive in petrol; in piping for domestic water; in sewage sludge waste and in agricultural products.

It has been known probably since Roman times that lead is a highly toxic cumulative poison following a long history of exposure to lead in food and drink. With the increased use of lead in industrial processes, many cases of lead poisoning were reported at work over the last century. Such frank poisoning cases - often following the ingestion of a large dose of lead salt - exhibited encephalopathy characterised by gross ataxia, vomiting, lethargy, stupor, convulsions, headaches, hallucinations, tremors and coma. Acute cases of this nature are rare in this country today, although chronic poisoning may arise following continued exposure to a series of smaller doses of lead over longer periods. The amount of lead that must be ingested to cause poisoning is influenced by a number of factors including the recipient species involved, individual susceptibility, age, water solubility of the source and the composition of the diet (Underwood, 1977; Humphreys, 1991). Many documented effects of lead on blood and its influence with the biosynthesis of haem, which is essential for the production of haemoglobin, have been recorded. In addition to anaemia, other symptoms of chronic lead

poisoning may include neurological defects, renal tubular dysfunction, aching of joints and abdominal colic (Harrison and Laxen, 1981; Hatch, 1982; and Quarterman, 1986). In general terms clinical poisoning is likely to occur at blood lead concentrations over 80 $\mu g100ml^{-1}$ and 50 $\mu g100ml^{-1}$ in adults and children, repectively, (Harrison and Laxen, 1981).

In recent times substantial attempts have been made to reduce lead in food, air, petrol,paint and water while soil lead abatement procedures have been introduced to reduce adverse health effects. Nevertheless there is growing concern that environmental exposure to lead in general terms may be harmful at concentrations much lower than those found to cause clinical symptoms. Almost fifteen years ago Needleman et al. (1979) reported that asymptomatic children with biochemical evidence of enhanced lead absorption displayed deficiencies on psychological testing and in classroom performance. Since then the long standing issue has been the lowest level of lead exposure at which adverse effects on health can be reliably demonstrated.

As a result, the question of lead and health remains an issue of current concern, with much research centring on the sources and pathways of lead in the environment and their effect on man and animals. The following study is based on a former lead mining area in Scotland which has remained largely unexplored.

1.2 LOCAL HISTORICAL BACKGROUND

This dissertation is an account of an investigation into the residual effects for man and young lambs of a former lead mining industry in South West Scotland. The first section sets the scene by examining historical factors, geology, environmental contamination, past research in the field and the case for further study.

The two former lead mining villages of Leadhills and Wanlockhead form part of a decaying industrial landscape set in the wildscape of the Southern Uplands of Scotland (Figure 1 a & b). It was this area, aptly named at the time as "God's Treasure House in Scotland", which formed the centre of the country's lead mining industry and which, at its peak in the late 18th to early 19th century, produced one tenth of the total UK lead ore (Porteous, 1876).

The first authentic records of mining date back to 1239 in Leadhills, but there is general agreement that there have been workings there since pre-Roman times with the local discovery of bronze and stone implements thought to be related to this particular period.

Although lead extraction has always been important, it was under the reign of James IV that orders were given to search for gold, and when the river gravels of the district were found to be gold bearing the extraction of this more valuable metal took precedent over lead mining. Nevertheless, lead mining is known to have been carried out on an intermittent basis up to the middle of the seventeenth century. It was however the 18th century when the lead mining industry began to progress steadily, prompted by the discovery of significantly rich veins and by an increasing demand for the metal both at home and abroad.

Prices for the smelted metal rose from £12-£13 per ton in 1720 to £32-£34 by the beginning of the nineteenth century. This greater demand and price was realised by the requirements of the Napoleonic Wars and by a marked improvement in technology, including improved drainage facilities and the introduction of steam engines.

Figure 1a. Leadhills Village

Figure 1b. Wanlockhead Village.

With the onset of peace, prices fell to £16 per ton but it was the introduction of the Free Trade Policy in 1832 which caused prices to fall still further with the cheap import of Spanish lead. As a result the industry suffered a distressing set-back which led to unemployment, bankruptcies and the abandonment of expensive steam pumping with over 200 miners and their families leaving the area.

It was not until 1860, when better prices prevailed and with the introduction of large scale capital investment and rail transport, that the industry saw a much needed revival. Four thousand tons of lead were produced each year between 1878 and 1895, providing 97% of all lead ore mined in Scotland - this despite operations being carried out at around 20 sites elsewhere.

From the beginning lead was sold abroad in the Low Countries, the earliest metal being sent abroad unsmelted as "Potter's Ore". By the 18th century lead was generally smelted and sold mainly to Holland and England, with subsidiary markets in France in the 17th century, in Russia in the late 18th century, in Germany in the nineteenth century, with occasional cargoes to the Mediterranean and China.

The lead ore and smelted bars were taken twenty miles by horse and cart to Biggar where there was a depot for lead, then to the port of Leith near Edinburgh before being shipped to the Baltic ports. At the turn of the 20th century a light railway running seven miles from the mining area to the main Caledonia line at Elvanfoot was constructed, allowing a somewhat easier export of the material.

In the years up to the first World War, the industry underwent fluctuating success, but in the aftermath of the war came a depression from which the industry never recovered. The high cost of pumping to provide adequate

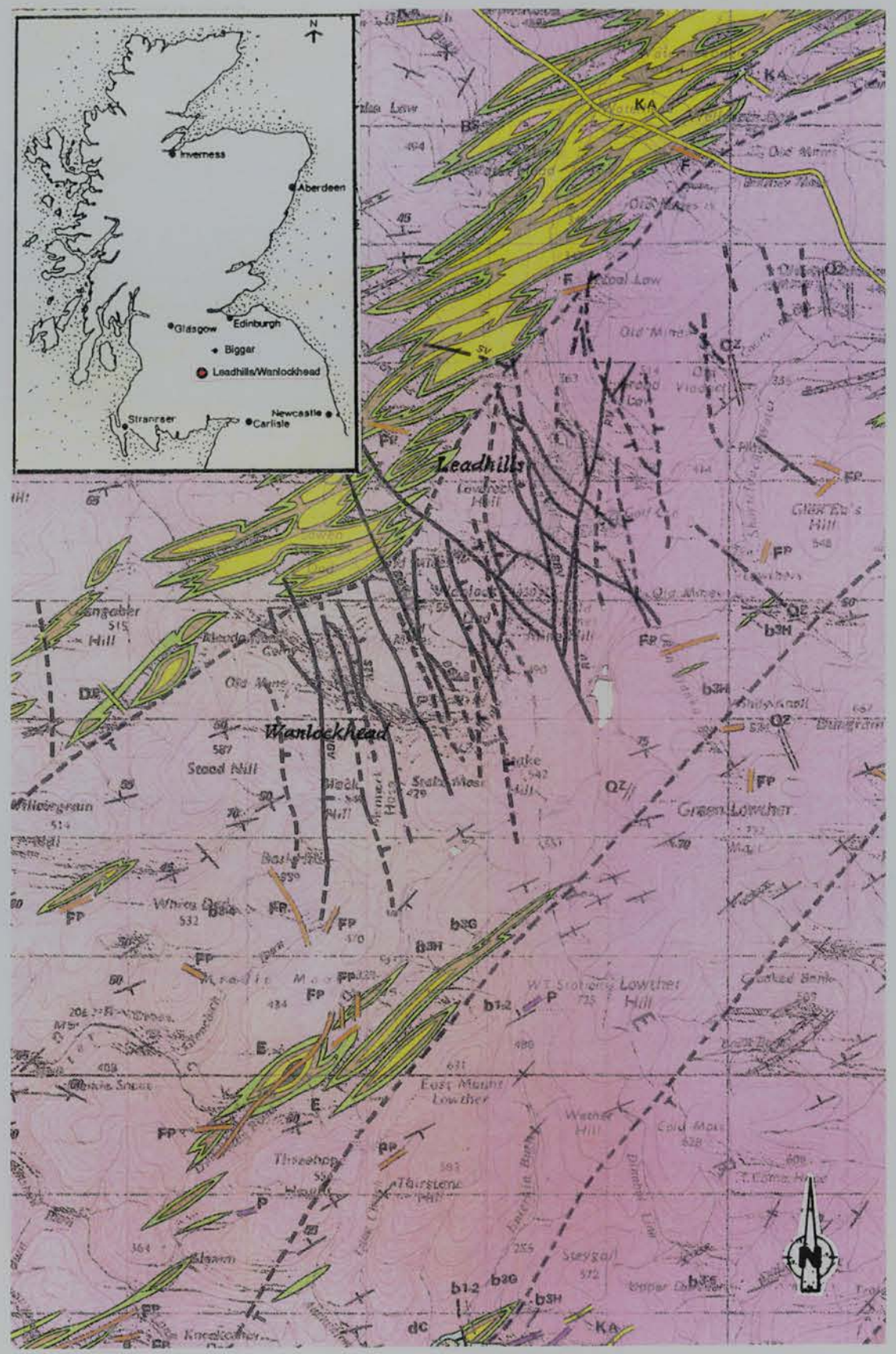
drainage and the low price of the metal due to cheaper imports led to the closure of the mines at Leadhills in 1928 and Wanlockhead in 1934.

The closure of what had been a very successful industry in the post war years gave rise to considerable unemployment. Many left the area and went south or abroad in search of work. Others stayed hoping that the mines would re-open but appeals for aid to the government were largely ignored and they too left. The depopulation of the area is highlighted by the population figures at the latter part of the 19th century which were approximately 2000 in Leadhills and 1200 in Wanlockhead. In 1992 the corresponding figures were 300 and 150, respectively.

Whilst geological surveys indicate that large reserves of lead remain, the extraction of further ore is not likely to be undertaken while there are cheaper sources abroad. Despite this, there are some optimists who still believe that as world reserves fall and prices rise, the probability of new mining will increase, although most think it unlikely.

1.3 LOCAL GEOLOGY

The Leadhills / Wanlockhead lead mining industry owed its existence to being situated in an area of Ordovician (Lower Silurian) rocks which have been intensely folded along NE-SW axes in the Caledonian orogeny (Figure 2). The general succession comprises volcanic rocks, successively overlain by radiolarian cherts, black shales, and greywackes of the Lowther Group. The volcanic rocks belong to the Arenig and the black shales and Lowther Group to the Caradocian and Ashgillian, respectively, (Pringle, 1948).



ORDOVICIAN – SILURIAN

Caradoc-Llandovery: interbedded greywacke sandstones, siltstones and mudstones with conglomerates

Conglomerate

ORDOVICIAN

Caradoc-Ashgill: interbedded greywacke sandstones, siltstones and mudstones with conglomerates. Palaeocurrent directions obtained from proximal lithologies indicate flow to south-east, while those from finer more distal lithologies show transport to north-east and south-west

Conglomerate

Llandeilo-Caradoc: interbedded greywackes etc., as Caradoc-Ashgill

Conglomerate

Lower Hartfell Shales: Black shales and mudstones with graptolite faunas ranging from *C. wilsoni* to *P. linearis* zones

Glenkiln Shales: Black shales, dark siliceous mudstones and cherts with graptolite faunas ranging from *N. gracilis* to *C. peltifer* zones

Arenig-Llandeilo: interbedded red and grey radiolarian cherts and mudstones

- Horizontal strata
- 40 Inclined strata, dip in degrees
- Vertical strata, long bar indicates strike
- Geological boundary
- As above, conjectural or position uncertain
- Fault at surface, crossmark on downthrow side
- T Reverse fault or Thrust plane
- K Unclassed basic dyke, underground
- Coal or ironstone outcrop
- QZ Quartz vein
- Metalliferous vein, crossmark on the hanging wall
- Ba Ba. Baryte

SHEET 15E

1:50 000 SOLID EDITION

Based on the First Series 1:50 000 Map parts of sheets 71, 72 & 78 dated, 1976.

Figure 2. Geological Map of Leadhills and Wanlockhead.

The outcrops of the three older groups are restricted to two NE-SW trending anticlinorial belts, one to the north of Leadhills and Wanlockhead, the other to the South. The intervening synclinorial area is occupied by the Lowther Group, a series of greywackes and shales estimated to be 1000 feet thick (Wilson, 1921), whose importance in the localization of the mineral deposits is demonstrated by the distribution of the mineral veins.

The greywackes of the Lowther Group vary from coarse breccias to fine shaly greywackes. The most important constituents of the rocks are angular or sub-angular quartz grains accompanied by plagioclase feldspar. Fragments of various rock types are common, including greywacke, slate, quartzite and fine-grained igneous rocks (Pettijohn, 1957).

The lead-bearing veins themselves are found in the belt of Silurian strata which stretches more or less continuously across the country from Port Patrick in the west to St. Abbs' head in the east. They are contained within an area some 2 miles wide, stretching for about 5 miles in a NE-SW direction parallel to the strike of the Country-rock. Most of the veins cross the strike at angles varying from 45° to 80° and can be divided into two main systems, one trending WNW to NW and the other NNW to NNE; a few thin quartz veins with a NE trend are also found. A peculiar feature of the veins is that most of them hade to the east, and of those that hade west fully 90% belong to the WNW-NW set.

Almost all of the veins contain galena (lead sulphide) as the principle valuable mineral. A few however carry ores of copper in excess of the other sulphides and have been worked for that metal. Most veins contain zinc-blende but it is usually not present in economically extractable quantities and has been worked only on a very limited scale.

Almost 70 veins are known to occur in the district, with the richest portion being found in the greywackes of the Lowther Group. They have well defined walls and vary in width from mere strings to 18 feet. They usually show typical banded structure, but quite often are filled with a breccia of greywacke fragments cemented together by a calcite or dolomite matrix sometimes containing interspersed galena. The whole vein is often cut by numerous vertical strings of ore varying from 0.5 inch to 3 or 4 feet in width, and in exceptional cases from 12-18 feet of solid galena.

The distribution of the veins and resulting mine shafts clearly indicate the original raison d'etre of the two communities, but as the next section shows they also led to widespread environmental contamination.

1.4 ENVIRONMENTAL CONTAMINATION

The legacy of past industrial activity and its resultant contamination of the environment is evident in today's landscape in the form of pits, shafts, scars on the hillsides, old tramways, smelters and the abandonment of waste materials. Perhaps most notable of all are the spoil heaps (Figure 3), the chemical and physical features of which are partly related to the parent rock, but more importantly to the extraction techniques and subsequent treatment processes applied to the lead ore.

The earliest method of lead ore extraction was by opencast working in shallow trenches along the line of the metal outcrop. At this time it was unusual for shafts to be sunk due to the problem of poor ventilation.

Figure 3. Spoil Material in the Contaminated Area.

Where possible, the miners avoided bringing rock to the surface and the mineral ores were hand-sorted and removed to the surface where a further separation process was carried out. Such a procedure was only partially effective in removing the metal and the resultant spoil heaps, although small in scale at this time, contained high, though variable amounts of toxic metals.

Gunpowder for mining was first introduced from Germany in 1670 but even by the mid-18th century this technique was not often employed because of expense. With the improvement of ventilation and drainage techniques and the introduction of rock drills and gelignite in the late 19th century came much deeper mining. Since the lead ore contained only 0.5 - 8% by weight of the valuable metal, vast quantities of waste material were produced.

It was however the treatment processes of the ore which led to widespread environmental contamination from the 19th century. In particular, the introduction of crushing stamps and washing plants to increase the extraction of metal added to the problems of pollution. Further pollution of the environment occurred through the technique employed for smelting the metallic ores. This process was initially simple and somewhat crude using a small blast furnace. However by the early 19th century the technique became more complicated and included water wheels, roasting furnaces and elaborate flues which ran zig-zag along the hillside for several hundreds of metres to allow lead fumes to condense out (Smout, 1967). Despite this, widespread pollution is known to have occurred (Moffat, 1982).

The extent of the spoil heaps is shown in Figure 4. Whilst there is a scattering of coarse waste dumps at both Leadhills and Wanlockhead, the fine grained tailings material from the washing plants is found concentrated at only one site in each village.

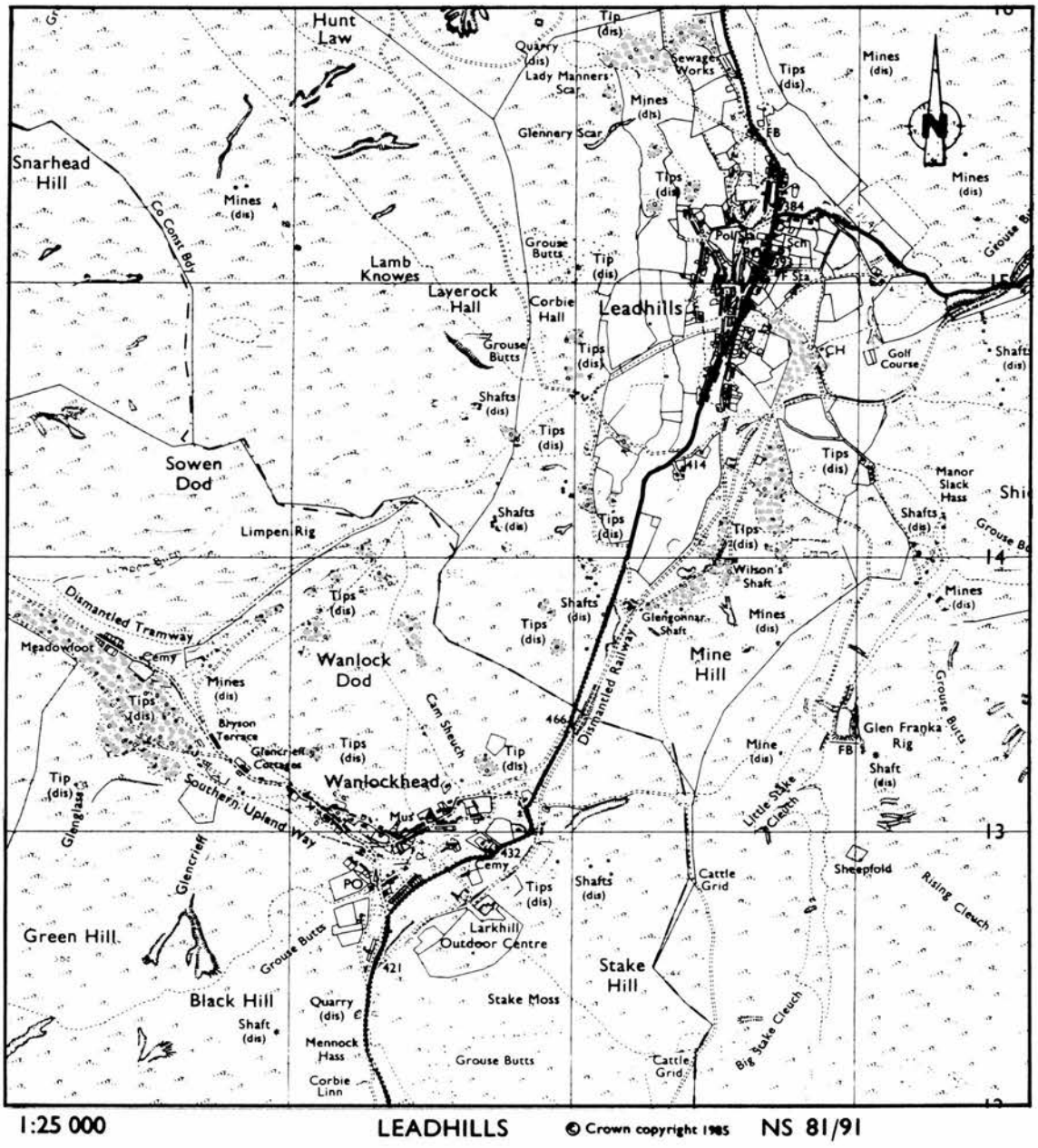


Figure 4. Geographical Distribution of Spoil Heaps in Leadhills / Wanlockhead.

Overall it is known that approximately 500,000 tonnes of mineralised waste is spread equally between the two villages with a lead content of between 1 and 5 percent indicating heavy metal pollution (Richards, Moorehead and Laing Ltd, 1985).

Due to a number of factors including lack of macronutrients, shortage of moisture and toxic levels of heavy metals including lead and zinc, the spoil heaps remain largely devoid of any vegetative cover (Smith and Bradshaw, 1970). The problem of environmental pollution is therefore heightened and confounded by the varying degrees of dispersal of spoil material through wind and water erosion. In addition, the river courses and flood plains associated with the former lead smelting sites are very heavily polluted. Overall, it is estimated that approximately 4000 acres of land are subject to some contamination, with local soils containing lead levels much in excess of reported norms (Moffat, 1982).

Perhaps the most obvious effect of environmental pollution can be seen in the sheep population of the area. A locomotor disorder in young lambs grazing land in the vicinity of former mining activity is attributed to lead poisoning by the local farmers. Although typical symptoms of lead poisoning per se are absent, the blood lead levels of grazing animals are known to be well in excess of the normal limit of 0.25 $\mu g ml^{-1}$ (Moffat, 1982). The condition is seen in young lambs between the ages of two and twelve weeks and occurs on six affected farms every year in the months of May, June and July.

The disorder is characterized by stiffness and slowness of movement, while the backs of affected animals appear hunched and 'rickety'. Brittleness of bones is the major disorder, causing multiple fractures which result in the lambs ceasing to play and following their dams dejectedly with small steps, showing a tendency to stand as if on

their toes. Stiffness of gait most seriously affects hind limbs although the fore limbs are also affected. Posterior paralysis may result when the lambs are subject to quick movement through herding or when suddenly startled (Butler et al, 1957). Losses from this disorder range between twenty and forty percent per annum in an affected flock, while a sizeable percentage of surviving lambs are affected to varying degrees and are slow to develop.

Once past what would appear to be the critical age of approximately twelve weeks, or if removed to uncontaminated pasture, most lambs recover. Upon return they may be kept on polluted pasture for up to five years without showing clinical symptoms. It is significant that ewes appear unaffected and lamb normally each year.

Other young animals may also be affected and having been brought up in the area concerned, the writer is all too aware of the problems which exist. For example, young dogs which drink repeatedly from pools of water lying on heavily polluted ground, exhibit typical symptoms of lead poisoning such as convulsions and stupor. Geese, hens and ducks are similarly affected by high lead levels picked up from the ground or sediment in streams when feeding.

The removal of toxic spoil material to sites outwith the area has led to clinical cases of lead poisoning in cattle and dogs. In two separate incidents three housed suckler cows and four heifers died with blood lead concentrations of between two and eight times the upper limit of lead in blood. The source of the problem was traced to mine waste taken from Wanlockhead which was used in the construction of a ramp over which silage was pushed (outbreak 1) and to fill potholes on the farm (outbreak 2). Two dogs have died, and others have been affected where mine waste was used to resurface a road (Wardrope and Graham, 1982). In 1989/90, two young dogs

which had suffered from severe convulsions were found to have blood lead values of 0.69 μgml^{-1} and 0.72 μgml^{-1} in Wanlockhead and Leadhills villages, respectively, almost three times the normal value. Moreover, a foal aged four months died, and was found to have fifty times the amount of lead considered normal in liver.

Apart from these isolated incidents in other animals the greatest death rate occurs amongst grazing lambs although it is by no means clear what the nature and extent of the link with lead is. There is no reported incident of lead poisoning in the human population of the area although concern has periodically been expressed regarding environmental lead levels and public health. To date, the issue of lead and human health in relation to lead from 'environmental sources' in Leadhills and Wanlockhead has not been studied.

1.5 CURRENT UNDERSTANDING OF EXPOSURE TO ENVIRONMENTAL LEAD

A wide variety of methods have been used to assess the degree of contamination of both man and animal's environment with lead, the most common being the analysis of blood samples to show the extent to which lead passes from the environment into the body. In this initial review it is acknowledged that not all studies show evidence of statistical significance or adequate analytical quality control, but having been developed around lead contaminated areas they may well show important trends.

1.5.1 Human Studies

Blood Lead Concentrations in Contaminated Areas

There are a number of studies in other former lead mining

areas of the UK which have looked at blood lead levels in the local populations. An illustration of this is given by Gallacher et al. (1983) who carried out work in an old lead mining area of Wales. There, mothers and their young children showed mean blood lead levels of 11.81 $\mu g100ml^{-1}$ and 22.58 $\mu g100ml^{-1}$, respectively, in an area where soil lead levels ranged between 140 and 10,181 μgg^{-1} Pb. Where soils were much lower in lead content and ranged between 21 and 280 μgg^{-1}, mothers had a lower mean blood lead level of 7.87 $\mu g100ml^{-1}$ and young children 17.61 $\mu g100ml^{-1}$.

Barltrop et al. (1975) determined blood lead levels in the inhabitants of Derbyshire's old lead mining villages and recorded geometric mean levels of 18.44 $\mu g100ml^{-1}$ and 24.86 $\mu g100ml^{-1}$ in mothers and children, respectively, in an area with soil lead concentrations between 1050 and 28,000 μgg^{-1}. In a considerably less contaminated area with soil lead concentrations of 130 μgg^{-1} to 3000 μgg^{-1}, mothers and their children had mean blood lead levels of 14.71 $\mu g100ml^{-1}$ and 20.93 $\mu g100ml^{-1}$, respectively. A study by Barltrop and Strehlow (1982) in the village of Shipham, Somerset where soils were mildly contaminated by past mining activity (a median concentration around 2350 μgg^{-1} Pb), reported mean adult blood lead concentrations of 10.15 $\mu g100ml^{-1}$: the control village of North Petherton had normal soil lead concentrations and mean adult blood lead levels of 10.77 $\mu g100ml^{-1}$. The mean blood lead concentration of children was 9.53 $\mu g100ml^{-1}$ in both villages.

The degree of contamination of soil in the above studies can be assessed by comparisons with various studies of uncontaminated soils. The Royal Commission on Environmental Pollution's Ninth Report on Lead in the Environment (1983), states that most soils in the UK contain lead in the range 10 to 150 μgg^{-1} and levels above 150 μgg^{-1} are most likely to have arisen from contamination. Nriagu (1978) calculates the mean in

natural soils as 17 μgg^{-1} Pb while Swaine's (1955) calculation of a normal range of 2-200 μgg^{-1} Pb has been widely quoted. More recent work by Davies (1983) in four areas of England and Wales, suggests the geometric mean content of lead for uncontaminated soils is 42 μgg^{-1} and that concentrations greater than 110 μgg^{-1} will not arise naturally.

Blood Lead Concentrations in Uncontaminated Areas

Published data for blood lead concentrations in rural areas of the British Isles not contaminated by past mining activity are scarce. Elsewhere studies have attempted to determine the 'normal' or baseline level of lead exposure by examining blood lead levels in remote populations. Piomelli et al. (1980) studied Nepalese natives living remote from industrialized areas, and showed adult males to have a geometric mean for blood lead of 3.73 $\mu g100ml^{-1}$; adult females 2.90 $\mu g100ml^{-1}$ and children 3.52 $\mu g100ml^{-1}$. Other research by Poole et al. (1980) indicated that a remote population of 100 children from Papua New Guinea had a mean blood lead value of 5.18 $\mu g100ml^{-1}$.

From the control populations sampled in the studies of three former lead mining areas cited above, it would appear that 'normal' blood lead levels in rural areas of this country are considerably higher than baseline, although it is worth noting that the 'low' soil lead area used in the study by Barltrop et al. (1975) with levels of up to 3000 μgg^{-1} Pb would be regarded as contaminated by most authors. Three further studies are perhaps more typical of uncontaminated rural areas in this country. Elwood et al. (1985) showed blood lead concentrations of the population of a small rural island situated seven miles off the north west coast of Ireland to be only 8.91 $\mu g100ml^{-1}$ for adult men, and 7.04 $\mu g100ml^{-1}$ for adult women, where no obvious environmental lead contamination

was present. Richardson (1982) found that children up to eight years of age attending schools in rural county Wicklow, Ireland, had a mean blood lead concentration of 7.04 $\mu g100ml^{-1}$. Strehlow and Barlop (1987) reported geometric mean blood leads of 6.37 $\mu g100ml^{-1}$ and 4.94 $\mu g100ml^{-1}$ in 5 to 7 year old children and mothers, respectively, from rural Suffolk, England in 1985. On a global scale, it is therefore arguable whether any population in or around the British Isles has totally escaped the consequences of lead contamination.

Decreasing Thresholds for Acceptable Blood Lead Concentrations

Over the past twenty years or so the acceptable upper limit for lead in human blood has been continuously examined and reduced in terms of health. By 1980, the Lawther Working Party of the Department of Health and Social Security concluded that there was indefinite evidence about the effects of lead at 'low' blood levels in the range of 35 to 80 $\mu g100ml^{-1}$, while others reported that blood lead concentrations previously considered safe at under 40 $\mu g100ml^{-1}$ could have harmful effects (Gloag, 1980).

Present government regulations in the United Kingdom and in the European Community now stipulate that where a person, particularly a child has a blood lead concentration greater than 25 $\mu g100ml^{-1}$ steps should be taken to reduce exposure (Department of the Environment and Welsh Office, 1982; D.H.S.S., 1980). In general, the EEC Directive 77/312 (D.H.S.S, 1980) further states a recommended maximum concentration for lead in blood of 35 $\mu g100ml^{-1}$ for 98 per cent of studied populations. Thus for two decades there has been increasing concern about metabolic and biochemical disturbances at levels of lead exposure previously thought to be entirely safe, with new data continuing to indicate the potential for adverse

health effects at blood lead levels currently held as acceptable. The overall effect of lead on haem synthesis and other enzymes at recognised 'low' levels under 25 and 35 $\mu g100ml^{-1}$ and subsequent sub-clinical effects are only part of the investigation.

Adverse Effects of Lead Exposure on Behaviour and Intelligence

The main argument at present centres on the blood lead level at which deficits in educational attainment, cognitive abilities and/or behavioural effects develop in the young. Controversy arises due to difficulties in ensuring adequate control of the confounding effects of covariates: these include socioeconomic variables of social class, parental intelligence, the home environment, nutrition, family size and sex. The evidence has been further complicated by differences in sample selection, different ways of allowing for confounding factors, small study population numbers, the variety of statistical analysis techniques employed, the measure of lead exposure employed (blood or teeth) and the method of cognitive psychological testing used. In reviewing this issue in 1983 the Royal Commission of Environmental Pollution reported that it was not possible to establish whether lead exposure per se influenced behaviour and intelligence.

Perhaps not surprisingly most concern today is centred on possible effects for young children. The reduction of lead exposure in children is now seen as being of paramount concern since young children can absorb up to 50 per cent of their intake of lead compared to around 5-10 per cent in adults (Chisholm and Barltrop, 1979; Bryce-Smith and Stephens, 1980; and Lenihan, 1983). In the last decade a number of child longitudinal studies have been reported from Europe, North America and Australia which deviate from earlier cross-sectional

research where lead levels and psychological development were measured at one point in time or were retrospective in nature.

Longitudinal studies based in Boston (Bellinger et al, 1986a, 1986b, 1987, 1989, 1990, 1991, 1992), Cincinnati (Dietrich et al, 1985, 1986, 1987, 1989, 1990, 1991, 1992; and Bornschein, 1985), Cleveland (Ernhart et al, 1981, 1987a, 1987b, 1988, 1990), Port Pirie (McMichael et al, 1985, 1986, 1988; Baghurst et al, 1985, 1992; and Wigg et al, 1988), and Sydney (Cooney et al, 1989a, 1989b; and McBride et al, 1987) investigate the relationship between low blood lead (means less than 25 $\mu g100ml^{-1}$) and mental development in young children. Each of the studies appear to have been designed and administered similarly through consultations, with multivariate analysis employed to account for confounding variables making comparison of results between studies possible. Prenatal and postnatal blood lead and neurobehavioural measurements are reported from birth, six months or annual intervals upwards of seven years. Prenatal and postnatal blood leads up to 57 months of age are reported as 0-25 and 0-25 $\mu g100ml^{-1}$ for Boston; 1-27 and 1-30 for Cincinnati; 0-15 and 0-30 for Cleveland; 9.5 and 6-57 for Port Pirie; and 0-29 and 0-29 for Sydney (Volpe et al, 1992). The Boston and Sydney studies analyse middle or upper class children and cohorts of 249 and 274, respectively; the Cincinnati and Cleveland studies inner city low income populations and cohorts of 292 and 260, respectively; and the Port Pirie study a working class cohort of 548 living near a smelter. Studies are ongoing in all of these towns apart from Sydney.

An inverse relationship between prenatal and postnatal exposure to lead and the Mental Development Index (MDI) was established in unadjusted analyses in all five studies. After controlling for covariates, a

statistically significant effect of prenatal lead exposure on the MDI was established at 12, 18 and 24 months in Boston. Similarly the Cincinnati group found a significant relationship between prenatal lead exposure and the MDI at 3 and 6 months, an effect which may have been partly due to an association between prenatal lead and birthweight, growth, and maturation. No significant associations were established between prenatal lead exposure and development in children from Sydney, Port Pirie or Cleveland, although the latter finding may be a result of population selection techniques.

Following control of covariates no postnatal effects of lead were found in the Cincinnati, Cleveland or Sydney studies. In Port Pirie 6 month blood lead was negatively related, though not statistically, to the MDI at 24 months of age, although the 48 month General Cognitive Index score was significantly related to 6, 24 and 36 month blood leads. In Boston, this latter score was similarly related at 57 months with the 24 month blood leads; intellectual and academic performance deficits at age 10 years were also related to 24 month blood leads in 148 children whose lead exposure and cognitive function had been previously assessed up to 57 months.

Nearly all these studies point to an inverse relationship between blood lead concentrations and some measure of psychometric intelligence or cognitive performance and show varying results possibly related to differences in the covariates and type of population employed. However despite early efforts to share information on the design and implementation of these studies it now appears there was no such agreement on the analyses and reporting of results. A recent attempt to pool the data from these studies using meta-analytic techniques was unsuccessful due to differences between studies in methods of reporting and analysing data (Thacker et al, 1992) resulting in no conclusive finding concerning the effect

of low-level body burdens of lead on IQ. Disbelievers of an inverse relationship between lead levels and decreased mental development suggest that any association might be confined to more socially deprived children. For example, Lee and Moore (1990) suggest that disadvantaged and less able children play outside more and so may ingest more lead from dirt, while more literate children are more likely to remain indoors reading. While this may be true, the fact that less able children play more outdoors may result in them being even less literate by encountering environmental lead. It may also be that if a parent(s) with an IQ lowered by exposure to lead have children, then controlling for parental IQ in such studies may result in over-control for lead exposure and therefore conceal a true relationship between blood lead in children and lowered mental developments.

At time of writing the concept of whether low level lead exposure below 25 $\mu g100ml^{-1}$ is associated with neurobehavioural deficits in children is still a matter of debate. Despite the diversity of opinion there is a growing body of researchers who believe that lead at low levels of exposure probably has a modest effect on young children which is consistent with a small, but biologically significant effect on IQ with levels of lead less than 25 $\mu g100ml^{-1}$ (Thacker et al, 1992). It is of course difficult to know whether any threshold of lead is safe, but large numbers in population terms may be affected even if the effect is small and there may be far reaching social and biological implications. Difficulties in distinguishing any effect of lead from the combined effect of numerous confounding variables will continue to pose problems through underadjustment and overadjustment and caution should be exercised in interpreting existing studies : a common strategy is still clearly required. Nevertheless, these studies enhance the importance of continued investigation on sources and pathways of lead in the environment.

Lead Accumulation in Hair

Of those studies in former lead mining areas of the U.K., only Barltrop et al. (1975) measured hair lead levels as a further indicator of body burden of lead. In the high and low soil lead areas of rural Derbyshire geometric mean lead values were 12.8 μgg^{-1} and 7.5 μgg^{-1}, respectively, in children (samples were not obtained from adults). Lead in the hair of 10 year old boys in five American cities was determined by Hammer et al. (1971): the geometric mean value was 57 μgg^{-1} in a city where the mining and smelting of lead was an important industry and some six times lower in a city primarily involved in education and agricultural trading. Taking a group which may be considered as normal, Klevay (1973) found geometric mean lead concentrations in the hair of Panamanians to be 12.1 μgg^{-1} in males and 18.6 μgg^{-1} in females, while Ahmed et al (1989) reported a normal mean concentration of 9.7 μgg^{-1} for 6-8 year old school boys. Research on hair lead by Chattopadhyay et al. (1977) gave a control rural group of 76 persons aged between 18 months and 73 years a geometric mean of 14.7 μgg^{-1} and 121 persons living near a lead smelter a geometric mean of 25.3 μgg^{-1}.

Hair lead concentrations are therefore increased by lead contamination of the environment but baseline values vary widely so that control populations in one study (eg. Chattopadhyay et al., 1977) can appear contaminated by comparison with other control groups (eg. Barltrop et al., 1975).

Lead in House Dust

Lead levels in house floor dust samples vary greatly in the literature but they do generally reflect the extent of contamination. In Wales, Davies et al. (1985) found no striking contrast between the old mining and control

areas although geometric means of 346 μgg^{-1} Pb and 169 μgg^{-1} Pb, respectively, were observed. On the other hand, lead levels in dust from the high soil lead area in Derbyshire (Barltrop et al, 1975) had a geometric mean of 1803 μgg^{-1} Pb, with a value of 565 μgg^{-1} Pb in the lower soil lead area. Away from former lead mining areas, Strehlow and Barltrop (1987) reported geometric mean levels over a three year period for rural Suffolk and central London of 333 μgg^{-1} and 857 μgg^{-1}, respectively.

Lead Contamination of Hands and Kitchen Surfaces

Several studies have implemented a 'wipe' method of measuring lead on hands as well as various household surfaces as an index of lead contamination. One such study carried out by Gallacher et al. (1984) in Wales, covered two areas with differing degrees of environmental lead contamination. Lead deposits on hands of mothers and their 1-3 year old children were 13.2 μg and 20.4 μg, respectively, in the old lead mining area, compared to 9.6 μg and 14.1 μg in a rural control area. Lead values from food preparation surfaces wiped in the old lead mining area of Wales were 13.5 μg compared with 10.0 μg in the control area. Results from two other control areas in Wales where traffic was the environmental source of lead were largely similar to the means cited for the rural control area. Work on hand lead by Roels et al. (1980) was done by rinsing 9-14 year old childrens' hands with a mild acid solution. Results for children in a rural school ranged from 11.4 to 17 μg compared to those in an urban school where a range of 12.7-20.4 μg was noted. In a school situated 2.5 km from a lead smelter a range of between 20.0 and 62.2 μg Pb was found while schools less than 1 km from the lead smelter had children with between 244 μg Pb and 436 μg Pb on their hands. Thus only mining or smelting activity caused a significant increase in the amounts of lead found on hands or kitchen

surfaces, reflecting perhaps the general effectiveness of personal and kitchen hygiene.

Lead in Domestic Water

A survey carried out by the DOE (1977) demonstrated that the majority of households in Great Britain had a lead concentration in day-time samples of tap water of around 10 μgl^{-1}. The level of lead in water will depend primarily on the presence or absence of lead piping, and the degree of hardness of the water supply. One such example in south west Scotland (Sherlock et al, 1982) showed water lead levels ranging from 10 to 1000 μgl^{-1} where lead plumbing was present and where there was water with a low pH. Thomas et al. (1979) found median levels of lead in day-time water samples of 560 μgl^{-1} in a housing estate with lead piping and 3 μgl^{-1} in houses piped with copper. In the study by Gallacher et al. (1984) water lead levels in the old lead mining area of Wales had a mean of only 4 μgl^{-1}, compared to the control area value of 3 μgl^{-1}.

Lead in Airborne Dust

Several studies have employed the technique of moss bags to monitor airborne dust lead. An investigation of environmental lead contamination by windblown material from a spoil heap at a derelict lead mine in West Wales, found a mean lead concentration in moss-bags of 6.1 (0.1-144.8) $\mu gg^{-1}day^{-1}$ and a mean annual background value of 0.6 $\mu gg^{-1}day^{-1}$ (Davies and White 1981). Work carried out in the Swansea area where lead pollution was known to exist, gave a mean of 2.11 (0.68-7.33) $\mu gg^{-1}day^{-1}$ (Goodman et al, 1975; Davies and White, 1981).

Considerable research has been done by the Department of Community Medicine, University of Dundee in the application of mosses to the measurement of atmospheric metal pollution (Gailey and Lloyd, 1983; Gailey and

Lloyd, 1986). For example, spherical moss bags were used to study the concentration of atmospheric pollution in the vicinity of a steel foundry in central Scotland (Gailey and Lloyd, 1986). Here, lead values ranged from a low of 0.14 $\mu gg^{-1}day^{-1}$ on the town's periphery to 5.01 $\mu gg^{-1}day^{-1}$ near the foundry over a 17 month period, with a mean figure of 1.11 $\mu gg^{-1}day^{-1}$.

Lead in Home-Grown Vegetables

Home-grown vegetables have also been considered as a source of lead contamination in man. A study of vegetables grown in the contaminated soils of Shipham found summer crops of cabbage and carrot with mean lead values of 0.46 μgg^{-1} and 0.49 μgg^{-1} and lettuce and potatoes which had mean lead values of 0.30 μgg^{-1} and 0.16 μgg^{-1}, respectively (MAFF, 1982). The same publication cites much lower levels for these four vegetables and others grown in a market garden environment: mean lead values for cabbage were <0.05 μgg^{-1}, carrots 0.08 μgg^{-1}, lettuce <0.09 μgg^{-1} and potatoes 0.04 μgg^{-1}. Research by Davies et al. (1979) and Davies and White (1981) also revealed elevated lead levels in vegetables grown on contaminated soils. Harrison and Laxen (1981) found a wide range of vegetables in the UK to contain between 0.04 and 0.26 μgg^{-1} lead: the higher values may well reflect contamination of the environment.

1.5.2 Lamb Studies

Disorders in Lead Polluted Areas

Butler, Nisbet and Robertson (1957) described the clinical, biochemical and histological findings of a lamb locomotor disorder in Leadhills and Wanlockhead on seven farms over a three year period. They reported that the principal feature of the disease was an excessive

fragility of the bones due to osteoporosis, which was responsible for locomotor disturbances ranging from a stiff gait to complete posterior paralysis. The most striking abnormality affecting half of the 32 lambs examined was a lesion involving one or more of the lumbar vertebrae. The cancellous bone in the lumbar vertebrae was deficient in amount and had a more delicate structure than normal. Many fractures, both recent and healed, were found in the bones most exposed to stress e.g. the ribs, metacarpal and metatarsal bones, humerus, femur and pelvis. These could result in lameness in the lamb, producing a bowed or knock-kneed appearance when the fore limbs were affected.

The overall condition was one of generalised osteoporosis i.e. the diminution of bony mass resulting from decreased production of osteoid tissue (bone matrix), but with normal calcification of the osteoid tissue which was laid down. The intact long bones of affected lambs when dissected out were more delicate and slender in appearance than those from normal lambs of the same age and breed.

Butler et al. (1957) pointed out that despite the lead content of blood from many affected lambs being unusually high (up to 2.50 $\mu g ml^{-1}$), the condition did not present a classical pathological picture of chronic lead poisoning. The normal blood lead value for lambs is less than 0.25 $\mu g ml^{-1}$. The only recognisable features of lead poisoning were present in those lambs with the highest blood lead values and took the form of basophilic stippling of the red cells, and acidophilic inclusion bodies in the liver and kidney. The only abnormality which was common to all affected lambs was a general deficiency of osteoid tissue.

It was concluded that the close association of the disease with pastures polluted by lead mining and

smelting suggests that it is caused by some toxic substance, originally present in the mineral deposits, which inhibits the metabolic activity of the osteoblasts either directly or indirectly by depriving them of some essential metabolite.

A similar disease in lambs has been reported from other former lead mining areas of the British Isles. Morgan (1924) described posterior paralysis in lambs grazing in the vicinity of lead mines in Cardiganshire along with chronic poisoning of ponies. Gardner (1924) reported lambs in similar districts in the North of England as being born with 'rickety' backs. Stewart and Allcroft (1956) also reported a disorder in young Swaledale lambs in the Pennines, characterized by a specific locomotor disability, stiffness and poor thriving. The flexor and extensor reactions of the affected lambs were sluggish, with the hocks and fetlocks underflexed so that the feet tended to drag along the ground. Such lambs, like those lambs grazing in Leadhills and Wanlockhead had abnormally high tissue lead values. A report by Clegg and Rylands (1966) from an area in North Derbyshire which used to be mined for lead describes an essentially similar condition, with lameness occurring as the result of fractures of fore or hind limbs. Paralysis was not found but post mortem examinations showed bones were fragile and easily cut or broken. Although symptoms typical of lead poisoning were not observed, these authors all considered that the condition was associated with the absorption of abnormal amounts of lead, with blood lead values ranging from 0.54 to 2.50 $\mu g ml^{-1}$.

There have been few studies of chronic lead poisoning in sheep in recent times. In experimental chronic lead poisoning of lambs fed a diet containing 200 or 400 $mgPbkg^{-1}$ in an acetate form, severe toxicity and deaths occurred with survival time related to the calcium, phosphorus and sulphur contents of the diet (Morrison et

al, 1977; Quarterman et al, 1977). Associated organ lesions have been found in experimental lead poisoning of sheep injected with 125 $mgkg^{-1}$ body weight of lead acetate, given in 20 equal doses administered every other day for 40 days (Kanakoudis et al, 1988; Vlemmas et al, 1988). None of these studies produced evidence of osteoporosis and locomotor difficulties.

Adverse Effects of Lead Exposure on Behaviour and Intelligence

Like humans, studies on behaviour and intelligence have been carried out with young animals, the brains of which appear to be more susceptible to the effects of lead during periods of swift development of the central nervous system. For example, a study of three groups of ten ewes and their offspring was implemented to determine if changes in learning capabilities on a visual discrimination task were present in the lambs of ewes ingesting subclinical levels of lead throughout gestation (Carson et al, 1974). Mean blood leads for ewes fed 4.5 (high) and 2.3 (low) $mgkg^{-1}day^{-1}$ of finely powdered elemental lead for five weeks and an unexposed control group were 34.8, 18.6, and 4.7 $\mu g100ml^{-1}$, respectively, during gestation. Lead exposure of ewes ceased at parturition. Mean blood leads for lambs from the 'high' and 'low' exposure ewes, and the control group at two to four weeks of age were 25, 17 and 6 $\mu g100ml^{-1}$, respectively; at ten to twelve weeks of age these figures were 14, 9 and 4 $\mu g100ml^{-1}$ lead. It was concluded that subclinical prenatal exposure to maternal blood lead levels of 34 $\mu g100ml^{-1}$ did slow learning of a visual discrimination task in lambs when they were 10 to 15 months old, although lambs exposed to maternal blood lead levels of 17 $\mu g100ml^{-1}$ did not differ from the controls. This may suggest a threshold of between 17 and 34 $\mu g100ml^{-1}$, for a neurologic effect of ewe blood lead

during gestation in sheep.

Other works have shown that lead acetate fed at 100 $mgkg^{-1}$ had no effect on the learning ability of five week old rats on a water T-maze, although 8 to 10 week old rats that had been nursed by lead exposed mothers for the first 21 days of life showed a reduced ability to learn a water T-maze Task (Brown et al, 1971; Brown, 1973).

1.6 THE CASE FOR FURTHER STUDY

1.6.1 Human Studies

Although much work has already been carried out on environmental sources and pathways of lead and their contribution to body burdens of lead, the previous section highlighting but a few, many studies have given inconsistent results and harboured design faults, making it difficult to assess the relative importance of environmental lead sources. There are a number of points worthy of discussion.

Sample Population

Of primary importance is the representativeness of sample populations. The risk of bias is determined by the response rates, that is, the proportion of randomly selected individuals which actually provide samples. A disturbing number of community-based studies fail to secure a random sample of subjects. An extensive study of exposure to lead in children living in the vicinity of a primary lead smelter by Roels et al. (1980) refers to control groups of age-matched children from rural and urban areas being examined but gives no indication of whether the selection was random or what response rates were achieved, while Angle and McIntire (1979) refer to child volunteers being recruited for their study. More

recently Brunekreef and Clausing (1987) mentioned low response rates of 50% for blood and 65% for environmental samples. Other low response rates for blood have been reported by Strehlow and Barltrop (1987) and Mahaffey et al. (1982)

Sampling Method

A further area of controversy concerns the choice of samples for analysis. For example, some studies have measured hair lead as well as blood lead (Barltrop et al, 1975). Much debate and conflicting evidence exists in the literature concerning the measurement of lead in hair, and its reliability, compared to that of blood as a measure of body burden of lead. A number of factors are in dispute the most important of which would appear to be whether hair samples should be washed prior to analysis to remove possible exogenous contamination. Furthermore, there appears to be no standard collection procedure. Further work is clearly necessary to investigate what relationship, if any, exists between hair lead and blood lead as indicators of lead exposure.

The relative importance of soils, house dust, domestic water, hand and household surfaces, and airborne dust as sources and pathways by which lead enters the blood in communities can be complicated to assess despite the large amount of data in the literature. Again a complication arises from the lack of a standard practice for taking samples. For example, some authors sample soil 0-15 cm in depth (Gallacher et al, 1984) while others use a 0-5 cm topsoil sample (Angle and McIntire, 1982). For household dust uncertainties arise because of differences in methods for collection of samples (Elwood et al, 1984; Laxen et al, 1988) and the size fraction of dust used for analysis. Interpretation of hand lead measurements may depend on whether hands were wiped (Gallacher et al, 1984; Sayre et al, 1974) or rinsed (Roels et al, 1980) to

remove the lead contamination: at present, the effects of the method applied on the end result are not known. The extrapolation from wipe methods to assess household surface lead is often complicated by a lack of information in studies on the actual surface areas investigated. Methods for sampling water from individual households varies from first flush (first water run out of the tap in the morning), to random day-time (first water out of the tap at time of visit), to running (after the tap has been running moderately for a stipulated time period) and stagnation samples (after no water has been drawn for a given time, for example 30 minutes). Much debate surrounds which method is most representative of a person's intake of lead from water (Thomas et al, 1979; DOE, 1977).

The restriction of some studies to certain environmental sources of lead could lead to an over-estimation of the importance of the chosen sources. Problems have not been resolved by repeated sampling of irrelevant sources, such as air lead on top of buildings (Nathanson and Nudelman, 1980) or in the middle of motorways (Colwill and Hickman, 1981) which are clearly not typical of the breathing zone of man.

Statistical Methods

Interpretation of data can be complicated further by the statistical analysis and presentation of results. For example, soil lead data normally have very wide ranges and are either reported as complete ranges (Culbard et al, 1983), including atypical results, or as the 95% confidence range having normalised the distribution by a suitable statistical transformation (Gallacher et al, 1983). The same is true for other environmental data including house dust and water samples. A common strategy is required to make full comparisons of data sets in all studies possible. It is also worth noting that few

studies state the reproducibility of their laboratory results lending doubt to the accuracy of published data.

Other Factors

Although some data exists for blood (Billick et al, 1979; Barltrop, 1979; Delves et al, 1985; and Baghurst et al, 1992), there is very little evidence in the literature for environmental sources of lead contamination being examined for effects of season. Limited research has been carried out on seasonal fluctuations in water (Matthew, 1981) and dust lead (Tsuchiya, 1975), and there is no agreement on seasonal influences from research on air lead (Tsuchiya, 1975; Billick et al, 1979; and Hwang and Wang, 1990). Overall, it would appear that seasonal changes in lead levels in the environment have not been extensively investigated.

Major authorities and reviewers have concluded that for the reasons discussed further information and research on the relative importance of the various sources and pathways by which lead enters man are required (MAFF, 1982; Royal Commission on Environmental Pollution, 1983).

Irrespective of any procedural deficiencies of previous studies of lead contamination of particular environments it would clearly be impossible to extrapolate from such studies to Leadhills and Wanlockhead since a host of factors will obviously differ between areas; for example the scale of contamination, the nature of lead waste disposal, rainfall and topography. These village communities have not been examined before and clearly merit a study of their own, where possible taking the experiences of others in the field into account.

1.6.2 Lamb Studies

Despite an indepth clinical report on the osteoporotic

disorder found in young lambs in Leadhills and Wanlockhead by Butler et al. (1957), there is no clear indication of the aetiology of the disorder. Butler et al. (1957) stated that while most of the evidence weighs against the incrimination of lead as the causative agent, no detailed study of chronic lead poisoning in lambs had so far been recorded. It was further pointed out that lead had never been known to cause osteoporosis.

Stewart and Allcroft (1956) investigated a similar disorder in lambs in the Pennines and measured blood lead levels in six ewes and four lambs from two affected farms over the first five weeks from birth. Lead values for blood from affected lambs, as well as for tissues and faeces were abnormally high but no control samples from unaffected lambs were taken in the study. These authors also state that although their results suggest that absorption of abnormal amounts of lead were associated with the disorder, 'the possibility that other factors are also concerned cannot be ruled out'.

Clegg and Rylands (1966) described similar clinical, histological and anatomical findings on affected lambs in Derbyshire. After post mortem examination of 7 lambs, the decision was made to treat affected lambs with ascorbic acid, 'in view of the comparisons which have been made between the skeletal lesions of lead poisoning and of scurvy'. The majority of lambs were given 200 mg ascorbic acid daily but a few were given 800 mg over a 4 week period. After a few days treatment the lambs were noticeably more active and moving more freely, the improvement being ascribed to the ascorbic acid. No control population of lambs was however employed in this study with no indication given on whether treated lambs remained on polluted pasture. The Vitamin C treatment continued to be applied on affected farms in Derbyshire (Farmers Weekly, 1979), but it would appear that when treated, improvement only occurs if lambs are removed

from lead contaminated ground (Aitkin P., 1985, Personal communication). There is clearly doubt concerning the validity of this work, since it is known that lambs in Leadhills and Wanlockhead removed from polluted ground also improve without being given Vitamin C.

It is now well recognised that the effects of dietary lead on animals greatly depends on the intake of a variety of substances other than lead. For example, the absorption and retention of ingested lead is notably affected by the dietary levels of calcium, phosphorus, sulphur, copper and zinc. Subnormal intakes of calcium, phosphorus and sulphur in lambs fed a high lead diet increased lead retention in body tissues, with dietary supplementation of minerals increasing lamb survival time (Morrison et al, 1977; Quarterman et al, 1977). Diets low or deficient in zinc increased lead absorption and tissue lead concentrations (Cerklewski et al, 1976) while increased lead levels may reduce blood and liver concentrations of copper (Hemmingway et al, 1964). Limited attempts were made to include such substances in the previous investigations in former lead mining areas: samples were taken for zinc and copper analysis in blood and tissues, and calcium and phosphorus in blood and bone by Butler et al. (1957); for copper in blood, liver and kidney and macronutrients in 'a few' serum samples by Stewart and Allcroft (1956); and for copper in blood and liver by Clegg and Rylands (1966). No consistent anomalies in these other elements were noted.

It is clear that the reason for locomotor disorders and death in lambs in former lead mining areas such as Leadhills and Wanlockhead remains unresolved. There has been no attempt to investigate the development of the disorder in depth over the first twelve weeks of life when lambs become affected, nor to make comparisons with a control farm population or to give detailed information on other heavy metals and macronutrients in the animals'

diet and environment. It is possible that findings in one species may help to resolve problems in the other: to date, studies in man and livestock have always been kept separate.

2. HUMAN STUDIES

2.1 DETAILED OBJECTIVES

A survey was conducted of blood and hair lead concentrations in the villagers of Leadhills and Wanlockhead, based on representative samples of male and female adults and of all children. Furthermore, possible sources of exposure to lead were investigated through the determination of lead in six environmental variables: garden soil, house dust, kitchen surfaces, domestic water, airborne dust and home-grown vegetable consumption. Hand lead contamination was also measured. Comparisons were made with a similar set of samples from a control village.

2.2 METHODOLOGY

2.2.1 Study Area

The investigations were centred in and around the villages of Leadhills and Wanlockhead in the Southern Uplands of Scotland on a typical upland moorland lying at an elevation of between 300 and 600 metres. The villages are notable for past lead mining activity as well as being Scotland's two highest settlements and lie in Clydesdale at O.S. grid co-ordinates NS 885150 and Nithsdale at O.S. grid co-ordinate NS 875130, respectively.

The principal road route through the villages, the B797, runs virtually north-south leading northwards to join the A74 Glasgow-Carlisle route and Southwards to the main Sanquhar-Dumfries trunk road. The main watercourse through Leadhills village is Glengonnar Water which flows generally from the south to the north, where it joins the River Clyde at Abington. In Wanlockhead, Wanlock water

flows westwards through the valley floor to join the Crawick Water (Figure 5).

The settlement patterns in both villages are similarly concentrated in a ribbon pattern along the narrow valley floors, with small terraces running sub parallel to the main line, hugging the valley sides. Most housing has existed for around 150 years and presents a unique picture in Scotland through the absence of post war and new private housing.

The indigenous population of Leadhills at time of study was approximately 270 with Wanlockhead around 130, giving an overall population of just under 400. However, the population of both villages swells considerably in the summer months and at holiday periods when at least twenty percent of houses are occupied as second homes.

Since the closing of the mines over 50 years ago, the main economic activity is that of sheep rearing on land owned by the Marquis of Linlithgow to the north of the area and the Duke of Buccleuch to the south. Although this means that most of the inhabitants have to seek employment outwith the villages, tourism has figured largely in the economy in the last few years, the attraction being the past mining era. The area boasts a museum, a guided tour of an old lead mine, examples of mining cottages refurbished in 18th and 19th Century style, the oldest subscription library in Britain and various monuments of interest. Excavations are currently under way to uncover further pieces of archaeological interest and a light railway is being reconstructed for recreational purposes between the old train stations in the two villages.

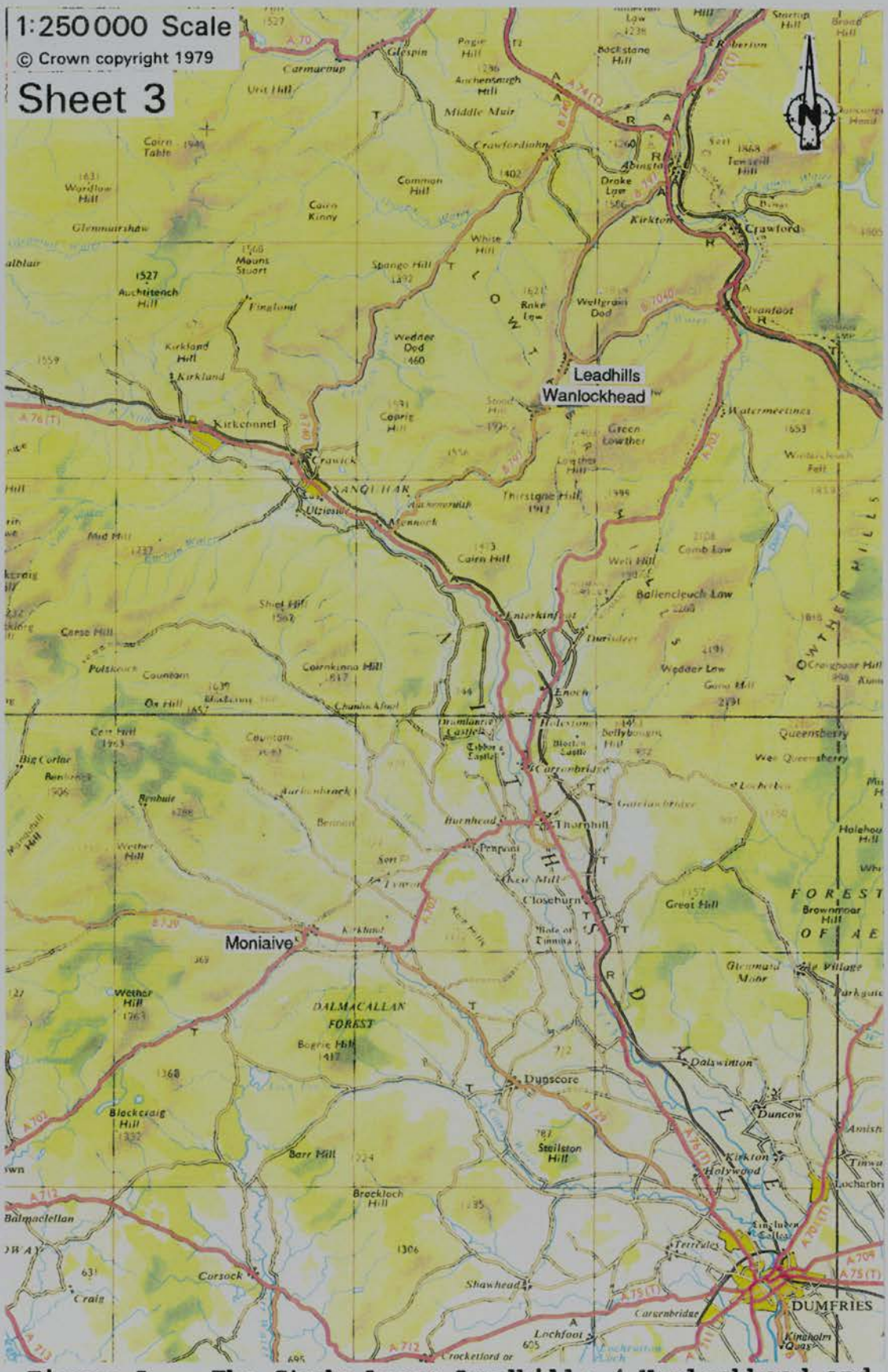


Figure 5. The Study Area: Leadhills / Wanlockhead and Moniaive.

After careful examination of the two council districts concerned, a control village Moniaive, was earmarked to serve as a comparison for the study (Figure 5 and 6). This village was chosen because it was as remote from heavily trafficked roads and industrial activity as Leadhills and Wanlockhead, and was similar in the most important respects other than having obvious signs of lead pollution. Moniaive has a population of around 420 and is situated 26 miles south-west of the study area in Nithsdale district (O.S. grid co-ordinate NX 780910). In terms of geology, the village lies in the same general belt of Silurian strata as Leadhills and Wanlockhead, but has no lead veins (Figure 7).

2.2.2 Population Sample

The names in the electoral registers for each village were placed in alphabetical order before drawing a sample of subjects. The first person selected for the study was determined at random, whereupon every third adult person was systematically chosen for inclusion from Leadhills and Wanlockhead. In Moniaive, the control village, a random one-sixth of the adult population was selected using the same technique as above. Young persons aged between 12 and 17 years were included in the 'adult' groupings for both areas.

In addition all those children aged under twelve not held on the Electoral Registers were included from each area since other research has shown young people to be particularly at risk from high levels of lead (Alexander et al, 1973; Barltrop et al, 1975). The numbers in this particular age group were so limited, (31 in Leadhills and Wanlockhead, and 19 in Moniaive) that use of a random sample would have introduced unnecessary error.

Figure 6. Moniaive Village.

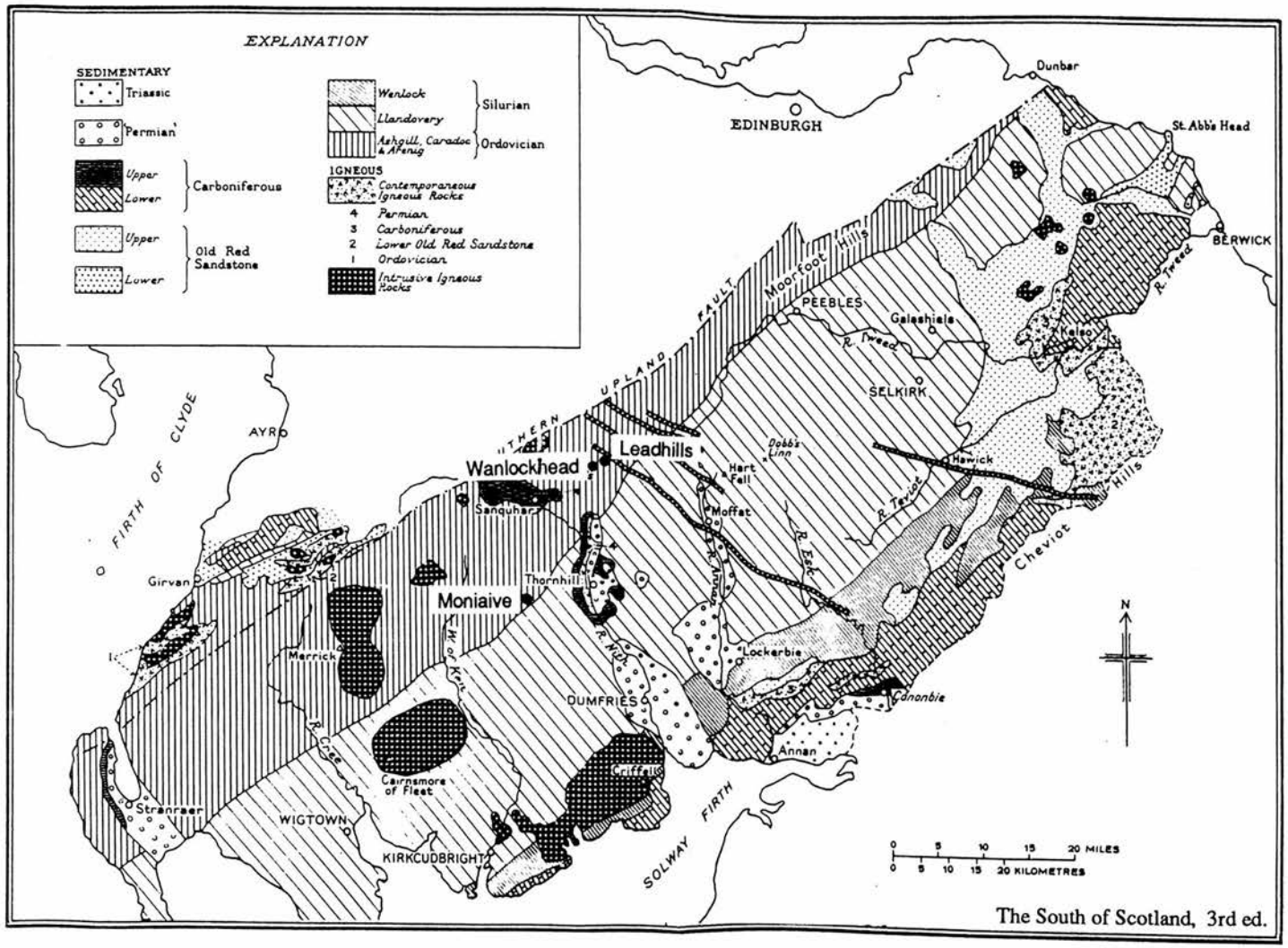


Figure 7. Simplified Geological Belt of the Study Area with Village Locations Earmarked.

In choosing the adult sample the main emphasis was on the provision of reliable estimates of the features of a representative population. Careful consideration was given to the planning of statistical investigations (Armitage and Berry, 1971, 1987) and to previous studies in the field (for example, Gallacher et al, 1984).

Prior to choosing the sample for participation in the study, co-operation was sought from the relevant local authorities, medical practices and community councils. Every subject was then visited by the author to seek their agreement to participation in the study, whereupon great care was taken to explain both the work involved and its objectives.

Where an adult individual refused to co-operate or had moved house, the person next on the electoral register was approached. Approximately 10 percent of the adult sample from all areas refused to co-operate but this was judged as insufficient to introduce any significant bias to the sample.

2.2.3 Investigational Techniques And Specimens

Environmental samples were collected from adults and households (including 12-17 year olds) on two occasions, firstly in February 1984 and again in June 1984. This was largely to check on the possibility of seasonal variation in lead status and stemmed from the knowledge that redistribution of spoil material occurs intermittently in the former mining area and could, therefore, play a part in the degree of lead contamination measured. In the summer months losses of water by surface evaporation and drainage can occur and an increase in wind blown dust has been observed.

Blood samples were taken twice as above for all adults drawn from the electoral registers and 12-17 year olds.

Young children aged 11 and under were sampled only once (in June 1984). Having obtained their agreement, letters were sent out to each individual requesting them to attend specially arranged blood clinics over an 8 day period on each occasion.

Blood

5 mls of venous blood were taken from 210 adults on each occasion and once from 37 young children by their own doctor and transferred to a medical tube containing the anticoagulant potassium EDTA. In every case the arm was cleaned with a medical swab prior to collection of blood to minimise contamination from the skin. On analysis, both the swabs and tubes were found to be free from lead contamination.

The blood samples were transported to the laboratory for lead analysis immediately after sampling. In order to avoid any bias, mixed batches of blood from the study and control areas were sent for analysis wherever possible. The laboratory was always unaware which populations were represented in the samples sent for analysis.

Blood lead concentrations were determined using a method based on that developed by Stoeppler et al, (1978). Samples stored at 4°C, were allowed to attain room temperature prior to analysis and then sonified on a Spiramix in acid washed tubes for 15 mins to break down any small clots which may have formed. A 200μl blood sample was taken to which 600μl deionised water was added and finally 200μl 25% Aristar nitric acid (BDH, Ltd) while agitating on a Vortex mixer. Tubes were then capped and spun at 1500 G for 15 min.

Treatment of the blood with a final concentration of approximately 5% nitric acid lyses the red cells and precipitates proteins and thus extracts lead from the

cells. After centrifugation, the clear supernatant was transferred to acid-washed autoanlyser cups and loaded onto the sample tray of a Perkin-Elmer 272 Atomic Absorption Spectrophotometer with an HGA 400 graphite furnace, ASI autosampler, and 056 recorder. Details of the furnace programme for the determination of lead in blood are described by Halls, (1984).

BDH Stock Lead Standard solution (1000 $\mu g l^{-1}$) was used in preparing working lead standards in the range of 0, 5, 10, 20 and 40 $\mu g 100ml^{-1}$. Standards for blood lead were prepared by adding known concentrations of lead to blood containing EDTA as an anticoagulant.

Results were calculated by constructing a calibration graph from the measured peak heights of the standards and by deducing the blank value before addition of lead (blood blank) against concentration of added lead ($\mu g 100ml^{-1}$). The peak height of the 'unknown' bloods was converted to a lead concentration from the graph. If there was any measurable peak height for a reagent blank (distilled water blank), this was deducted from the peak height of the unknowns before reading the concentration from the graph.

Stringent quality control was exercised and gave low coefficients of variation within and between batches of 2.2% and 4.1%, respectively. As part of this five samples were sent for analysis every week to the Supraregional Assay Service (SAS). These 5 samples were run with 3 previous SAS quality control samples. One sample per month was supplied for analysis under the UK National Quality Control Scheme. The results of these and their significance are explained later.

Throughout the lead analyses, control of possible contamination from external sources was essential. Therefore, all glassware, pipettes, micropipette tips and autosampler cups were washed with 20% v/v nitric acid and

then rinsed with distilled water before use. Gloves were worn at all times by the analyst to avoid contamination from perspiration.

Hair

Hair samples (246 in all) were taken simultaneously with blood at the February and June clinics as an non-invasive indicator of body burden of lead. These were obtained using latex medical gloves and stainless steel scissors from hair at the point of attachment to the nape of the neck. Hair from an area nearest to the scalp (2-4 cm in length and 0.5 cm wide) was retained in a sealed polyethylene bag for analysis. Sample weights ranged from approximately 0.04 g to 0.12 g dryweight (DW).

Facilities for a rigourous wash procedure prior to analysis were unavailable and results for the unwashed samples will therefore give an overall indication of internal and external lead levels in hair.

Equipment for use in the analysis of hair samples and all analyses described in subsequent methodologies was steeped overnight in 5% nitric acid, and rinsed three times using distilled water where appropriate.

Hair samples were carefully removed from each bag using sterile plastic forceps and placed in individual 100 ml Pyrex beakers for overnight drying in an oven at 80°C or until constant weight was achieved. Having determined the dry weight of each sample, 25 ml of BDH Spectrosol 70% nitric acid was added to the 100 ml beaker and the samples were digested on a hot plate. Each specimen was taken to near dryness and subsequently taken up in 20 ml of 0.1 N nitric acid and filtered through No.4 Whatman filter paper. Resultant solutions were made up to 50 ml volume using distilled water. Reagent blanks (2 for every 30 samples) underwent a similar process.

Lead estimation was determined in the aqueous digest using a Varian AA-1475 series Atomic Absorption Spectrophotometer fitted with a GTA-95 carbon furnace attachment, a wavelength of 283.3 nm and a hollow cathode lead lamp (Juniper Ltd). A duplicate injection was made of each sample solution. Results were interpreted from a calibration graph against standards in the range of 0.8, 1.6 and 3.2 $\mu g g^{-1}$ Pb which were prepared from a BDH stock standard solution as before.

Environmental Samples

Garden Soil

Total lead concentrations in soils were monitored in a random third of households participating in the study, totalling 48 samples in all. Soils were normally collected from the rear garden of the house, (usually from a vegetable garden) although in a very few instances this was not possible and soil was taken from the front. Five samples were collected from each garden using a stainless steel trowel to a depth of approximately 15 cm (Gallacher et al, 1984; Davies, 1983) and then bulked and sealed in polyethylene bags to await analysis.

In the laboratory, each bag of soil was mixed well, spread out, and allowed to dry in air at room temperature. The air dry soil was then ground gently using a roller mill until it passed through a 2 mm sieve. A 4 g sample was placed in a 100 ml Pyrex beaker on a steam bath. Digestion was achieved by adding two successive 30 ml aliquots of aqua regia* (*prepared by mixing 6M hydrochloric acid and 16M nitric acid, in the ratio 3:1) and 20 ml of 6M hydrochloric acid, taking to dryness after each addition. The residue was then treated with 15 ml of 1.5M HCl and digested under a watch glass on the steam bath for 30 minutes. The resultant extract

was filtered into a 50 ml Pyrex volumetric flask through a 9 cm, No.40 Whatman paper previously soaked in distilled water. Both the beaker and filter paper were given several washes using hot distilled water before making up to the 50 ml volume once the filtrate had cooled to room temperature.

Standards were prepared from BDH stock lead standard solution (1000 μgml^{-1}) by serial dilution with 0.5M HCl to contain 0.5, 1, 2, 4, 8, 16, & 32 μgml^{-1} Pb. All standard solutions were thus prepared using matched HCl acid concentrations. Lead determinations were made using a Pye Unicam SP9 atomic absorption spectrophotometer equipped with a single-slot burner and an air-acetylene flame. A hollow cathode lead lamp (S. and J. Juniper and Co, Harlow, Essex) and a wavelength of Pb 217 nm was used. Results were calculated from a calibration graph as previously outlined.

House Dust

As a measure of the concentration of lead in settled house dust, 153 samples were collected in February and June 1984 from the vacuum cleaner bag of every household participating in the study. Samples taken in this way were felt to be fairly representative of an individual's exposure to lead in house dust on the assumption that most time is spent in the living room, and that room will be vacuumed most often.

Dust samples were mixed and passed through a 1 mm aperture sieve. 5 g of this fine dust was weighed into a 50 ml Pyrex beaker, dried in an oven at 100°C for 24 hours, reweighed and ashed at 430°C in a furnace for 24 hours. Once cool the ashed dust had 20 ml of nitric acid added, was covered with a watch-glass and heated on a hotplate at 110°C for 30 minutes. The cover glass was then rinsed into the beaker and removed and the contents

of each beaker taken to near dryness at a temperature of 120°C. Care was taken to avoid any 'baking' of the sample; if baking occurred the above procedure was repeated.

To the 'dry digest' of dust, <50 ml of 0.01 M nitric acid was added and the sample broken and stirred with a glass rod, the latter being rinsed with a few mls of acid. Samples were then warmed on the hotplate at 50°C for 15 minutes and filtered through a fluted Whatman No.540 filter paper into 50 ml volumetric flasks and made up to the mark with distilled water. Standards containing 1, 5 and 10 $\mu g ml^{-1}$ Pb were prepared as dilutions from BDH 'Spectrosol' lead nitrate solution. Lead was determined using a Pye Unicam SP2900 Atomic Absorption Spectrometer with an air acetylene flame and a wavelength of 217.0 nm (Davies et al, 1985).

Hands and Kitchen Surfaces

As an indication of the possible transfer of lead from hands and kitchen food preparation surfaces to the individuals concerned, each person was asked to wipe their hands thoroughly at the end of a normal day before washing using a 'Boots baby wet wipe' paying particular attention to the nail areas. A total of 239 samples were obtained on two occasions. In order to ensure uniformity of sampling, copious explanatory notes were distributed to every household a few days prior to each blood clinic detailing how samples should be taken. These were given out on a personal basis, with time allotted to every individual to ensure understanding of the basic technique. Wipes were placed in a plastic bag labelled HW with a personal code number. In households with children aged under 12 years, a parent was asked to clean his/her hands first as above and then clean the child's hands thoroughly with a fresh wet wipe, again paying particular attention to the nail areas. Where more than one child

was involved, the parent's hands were cleaned in between times with a fresh wipe which was to be disposed of before cleaning a second or third child's hands. Personally numbered bags were again allocated.

Having cleaned all hands, the same adult was requested to use the remaining wipe to clean a kitchen food preparation surface approximately 1 metre square, and enclose this wipe in the small plastic bag labelled KW (153 samples). All samples were then returned to the blood clinic.

Each hand wipe / kitchen wipe was removed from the plastic bag using sterile plastic forceps, placed in a 100 ml Pyrex beaker and dried overnight at 80°C. Samples were then weighed and 25 ml of Spectrosol nitric acid added prior to acid digestion on a hot plate. The digested wet wipes were taken to near dryness, filtered through Whatman No.4 paper and made up to 100 ml using distilled water. Both the beaker and filter paper were rinsed several times throughout this process. As with garden soil samples, the standards for analysis were prepared from BDH Stock lead standards solution (1000 $\mu g ml^{-1}$) but at the lower concentrations of 0.2, 0.5, 1.0, 2.0 and 5.0 $\mu g ml^{-1}$ Pb. Two method blanks were included in each batch of 30 wipes analysed, using two unused wet wipes. Any reading obtained for presence of lead in the blanks was subtracted from every sample. Reagent blanks were also run. Analysis was carried out using a Pye Unicam SP9 Atomic Absorption Spectrophotometer.

Domestic Water

157 first flush and 157 random day-time samples of water were taken in February 1984 from the cold tap of every household where a person contributed blood. In June 1984, only a random day-time sample was taken from 1 in 3 of the original households tested. For households of young

children aged under 12, a first flush and random day-time sample was obtained in June 1984 from the households involved.

Acid-washed, polyethylene bottles were hand-delivered to every house, along with explanatory notes on how each sample should be taken the following day. 'First flush' water was drawn from the cold tap first thing in the morning into a 1000 ml bottle before any water was run from the system. Random day-time samples were drawn into a 250 ml bottle at some undetermined point during the day, but always after some water had run through the system. All bottles were collected on the day of sampling and delivered to the laboratory. Prior to collection, the laboratory ran a random sample of bottles to check for lead contamination, which proved satisfactory in all cases.

On arrival at the laboratory, samples were acidified using 8 ml Aristar hydrochloric acid l^{-1} to minimise the adsorption of lead onto the container walls, while awaiting analysis. Samples were concentrated 10 times by evaporation in Borosilicate glass beakers on a hotplate without boiling, adding more sample as the evaporation progressed, until only 10 ml remained. 1 ml of Aristar hydrochloric acid was then added and the solution made up to 25 ml with distilled water. Samples were aspirated into a Varian 1275 atomic absorption spectrophotometer using a four point calibration curve of 0, 1, 4 and 10 $\mu g ml^{-1}$ Pb, prepared from BDH Stock lead standard solution, the method overall following the guide-lines set by the DOE, 1980.

Airborne Dust

Sophisticated equipment for measuring air lead such as high volume air samplers and dust deposit gauges were not available. However, the 'moss bag' technique pioneered by

Goodman and Roberts (1975) and used in various other studies (for example, Davies & White, 1981) was used as an inexpensive way of obtaining data for airborne dust lead contamination.

Sphagnum spp. was collected from an uncontaminated remote upland moorland site sixteen kilometres from the study area, and was chosen for its high cation exchange capacity and known affinity for heavy metals such as lead, as well as its excellent water retention properties (Little and Martin, 1974; Clymo, 1963). The moss was collected and then hand-sorted with dead or dying tissue discarded and only healthy fonds picked for use in the study. It was then washed in O.I.N HNO_3 and rinsed three times in distilled water. Washing in a mild acid solution leaches out all of the metals held by cation exchange in the living moss and therefore leaves all sites available for binding with any air contaminants, particularly lead. An additional advantage of acid-rinsing is that having arrested metabolic activity, any differences experienced in the results are not likely to be due to differences in the metabolic activity of the moss (Goodman and Roberts, 1971; Gailey, 1983). It is further noted that the uptake of metals into mosses is predominantly by passive mechanisms (over 90 percent) and thus only a small reduction in metal uptake occurs by using acid-washed moss.

Small spherical bundles of air-dried moss approximately 4 cm in diameter were enclosed in nylon hair nets in a loosely packed form. Each bag was then suspended freely from bamboo canes two metres above the ground using plastic covered wire and thoroughly moistened with deionised water before exposure. Moss bags were randomly positioned in the gardens of 30 households in Leadhills and Wanlockhead, with a further 10 acting as control in Moniaive (Figures 8 and 9).

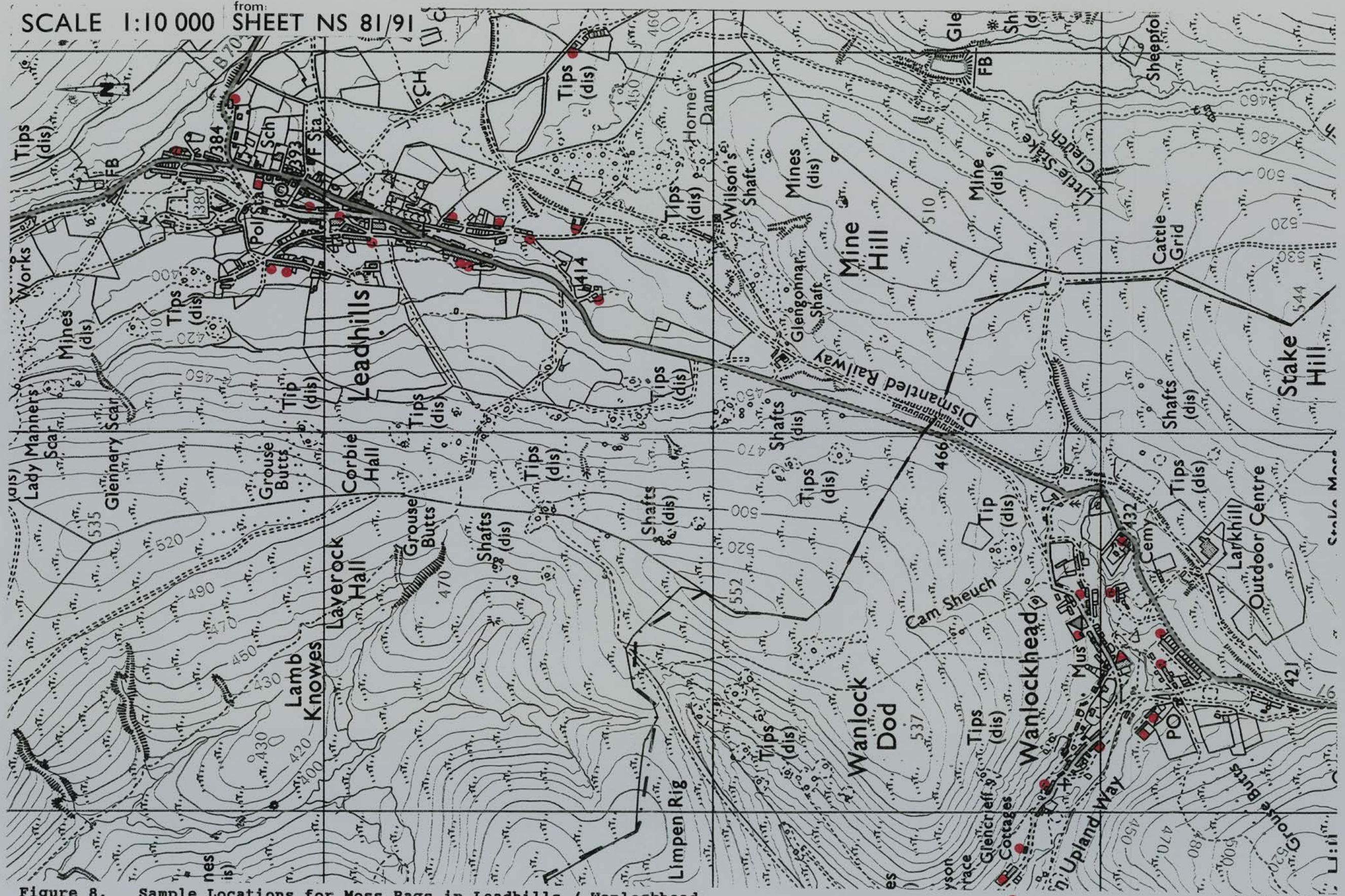


Figure 8. Sample Locations for Moss Bags in Leadhills / Wanlockhead.

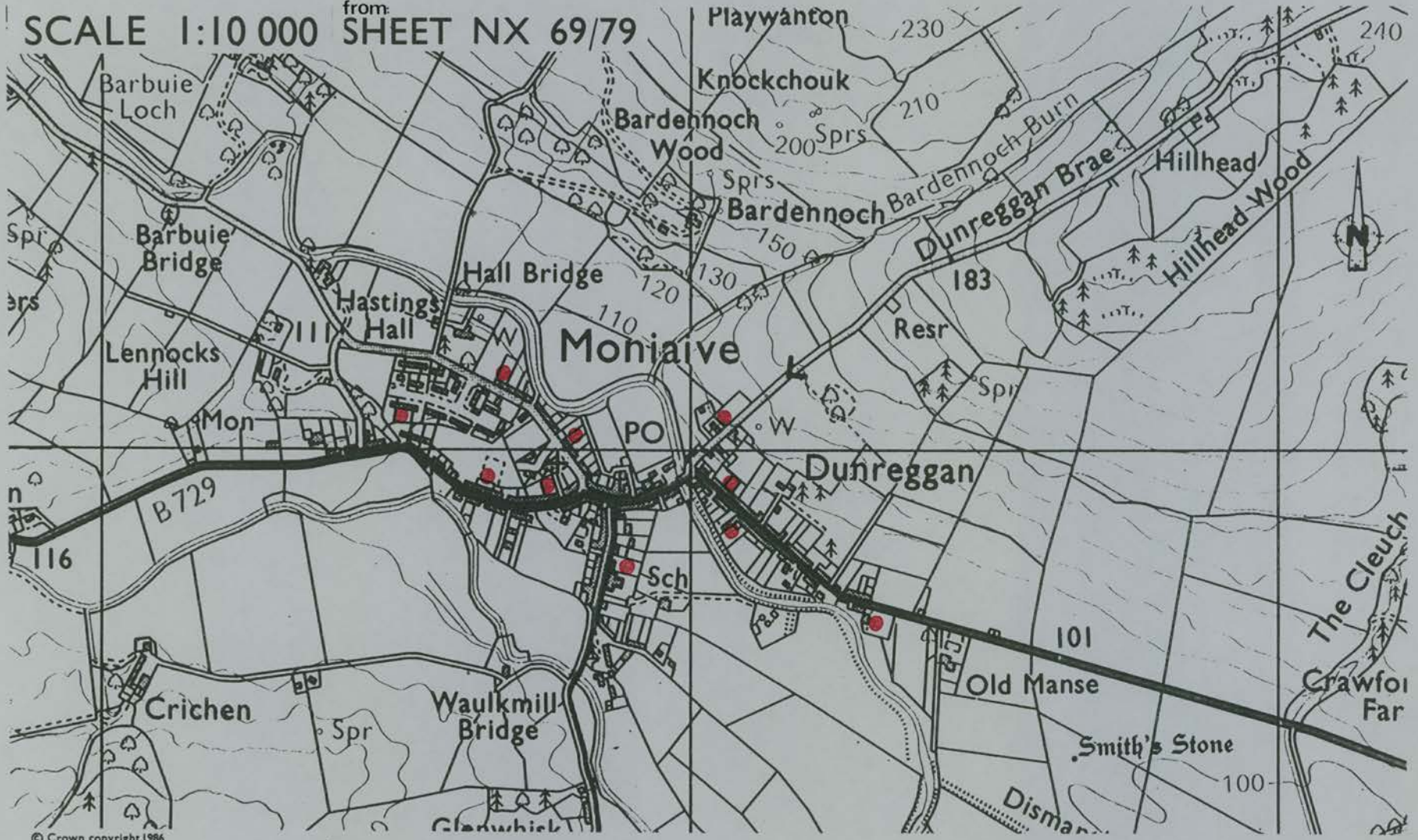


Figure 9. Sample Locations for Moss Bags in Moniaive.

Metal uptake by the moss is greatly influenced by moisture, and so in order to obtain a representative picture throughout the year, each moss bag was replaced every 30 days or so. The exposure period was limited on the basis of previous work (Goodman et al, 1975) to avoid saturation of all the ion-exchange sites occurring.

Initially set up in March 1984, the moss bag sampling continued until early December 1984 (frozen ground conditions prevented placement of bamboo canes prior to March). Although the moss bags were hung at a height which might reflect the 'breathing zone' of most individuals, a more precise measure might theoretically be obtained by hanging moss bags indoors in a living room, where most time is likely to be spent. However, despite excellent co-operation by the participants in the study in all other areas described, this idea met with considerable resistance.

Every month the 40 moss bags were collected for analysis along with nine other samples at sites randomly chosen to provide duplicates on two occasions throughout each study area, as a check on reproducibility of the technique. Recovery rates were high at around 95%. Using gloves and sterile forceps, each moss sample was removed from the nylon hair bag on return to the laboratory, placed in a 100 ml Pyrex beaker, dried overnight at 80°C and weighed. 25 ml of Spectrosol nitric acid was added to each beaker and placed on a hot plate in order to digest the mosses. Samples were taken to near dryness, filtered through Whatman paper No.4 and made up to 50 ml in volumetric glassware with distilled water.

As well as running reagent blanks with each batch of samples, two method blanks with unused moss underwent a similar process to determine background levels of lead in the moss. Using standards made from BDH Stock lead standard solution (1000 $\mu g \mu l^{-1}$) to contain 0.2, 0.5, 1.0,

2.0 and 5.0 μgml^{-1} a calibration graph was constructed using a Pye Unicam SP9 spectrophotometer as outlined for garden soils. Background levels of lead present in the moss before exposure in Leadhills, Wanlockhead and Moniaive were subtracted from the result for each exposed moss bag. These values were on average 8 μgg^{-1} and ranged from 4-15 μgg^{-1} Pb.

Home Grown Vegetable Consumption

In the course of collecting information for each study population such as sex and age, individuals were questioned closely concerning their home grown vegetable consumption, in an attempt to determine if this could be a significant factor in contributing to variation in blood lead levels. A record was constructed for all 247 individuals as to the proportion of vegetables eaten which had been grown at home. This information was categorised according to the following three groups: (1) all or most (2) some (3) none

Vegetable Lead Levels

It was not the intention of this research to relate lead concentration in vegetables to blood lead levels, as this would have involved a large detailed sampling programme of the many types of vegetables in order to produce a comprehensive result. The objective was to establish the amount of lead in a limited number and type of vegetables. A small number of samples were therefore randomly collected in late June, 1984 from the gardens of a sub sample of those involved in the study and confined to the four commonest vegetables, potato (n=30), cabbage (n=20), carrot (n=21), and lettuce (n=18).

In every case, the vegetables were removed from their individual polyethylene bag, hand-sorted, washed thoroughly, and in effect prepared for analysis as the

housewife would for food consumption. The coarse outer leaves in both the cabbage and lettuce samples were therefore discarded. Carrots were peeled, although potatoes were left unpeeled as being 'new' from the garden it was judged that this was the form in which they were most likely to be eaten. In order to avoid possible bias in this process, the preparation and washing of the vegetables was carried out by someone unconnected with the study at the author's request.

Vegetables samples were sliced thinly using a stainless steel knife, placed in 150 ml Pyrex beakers and dried until constant weight was achieved at a temperature of 80°C. Thereafter the dry material was ashed in a carbolite muffle furnace at 430°C, and digested over a hot plate using 30 ml of Spectrosol concentrated nitric acid. Taken to near dryness, the samples were filtered through Whatman No.4 filter paper taking care to rinse the beaker and paper with distilled water several times, and finally made up to 50 ml volume. Two reagent blanks were run with each batch of 30 samples. The range of standards employed and the final method of analytical detection are as outlined for garden soils.

2.2.4 Analysis, Sampling And Control Of Bias

Laboratory analysis was carried out under the direction of various establishments and personnel. Blood analysis was undertaken by G.S. Fell, D.J. Halls and staff of the Biochemistry Department at Glasgow Royal Infirmary; house dust was analysed by K. Paveley, under the guidance of B.E. Davies, in the Geography laboratories of Aberystwyth University; and water samples were assayed by Solway River Purification Board, Dumfries.

All other samples - garden soils, hand wipes, kitchen surface wipes, airborne moss bags, vegetables, and hair samples - were analysed by the author. The first four of

these were carried out in the University of Edinburgh's Geography Department laboratories, with hair being analysed in the Trace Element Laboratory of the East of Scotland College of Agriculture.

Where possible, at least 1 in 10 samples were run as 'blind' duplicates as a check on reproducibility of laboratory estimations. For some samples this was not feasible; for example, hand and kitchen wipes where accurate duplication was impossible and for hair, where the weight of sample was not sufficiently high to split a sample. In addition to reagent blanks, certified and inhouse reference materials were also applied wherever feasible as a further check on quality control.

The control of possible bias throughout this study was viewed to be of paramount importance. As part of this, stringent controls were placed on samples such as blood, dust and water which were required to be sent to other analysts. A coding system was implemented so that these analysts were totally unaware of which area the samples had been collected from. For laboratory work undertaken by the author, a coding system and re-labelling exercise was developed by secretarial staff so that until the analysis work was complete, the origin of the samples was unknown.

2.2.5 Statistical Methods

In order that parametric statistical tests could be applied, the cumulative frequency of the variables in each data set were plotted on normal probability paper in an attempt to determine if each set of variables was normally distributed. This showed that only the blood data were normally distributed. Hair, hand, soil, house dust, kitchen surface and airborne dust lead data were found to be skewed data sets, and were transformed to their common logarithms to yield the 'best' straight line

when the cumulative frequency of the transformed variable was plotted on normal probability paper. Water lead levels were also found to be skewed and in this case a cube root transformation was employed to give a normal distribution.

A series of Student 't' tests for significant differences between populations was carried out and Pearson's correlation analysis used to determine relationships between the different variables. Finally, step-wise regression analysis was applied in an attempt to evaluate the contribution of each environmental lead source to blood lead.

2.3 RESULTS

2.3.1 Response Rates

Response rates for the study are presented in Table 1. The response rates were high overall, averaging 95% for men, 98% for women, though understandably lower (75%) for children in the two areas. Of those adults first sampled in February, 87% from the contaminated villages and 83% in the control area, donated blood for a second time in June 1984.

Table 2 gives the number of samples for the different variables - Blood, Hair, Garden Soil, House Dust, Hand Wipe, Kitchen Wipe, Domestic Water and Airborne Dust - measured in Leadhills and Wanlockhead, and in Moniaive, the control village. Unless otherwise stated, all the following tables represent the mean for data collected from February and June.

TABLE 1

Population samples - numbers and response rates in the contaminated villages and the control village.

	LEADHILLS/WANLOCKHEAD			MONIAIVE		
	Men	Women	Children	Men	Women	Children
Number available	171	187	31	194	203	19
Sample number drawn	57	73	31	46	41	19
Number sampled	55	71	22	43	41	15
Response rate	96%	97%	71%	94%	100%	79%

Note: Figures for men and women are the mean for February and June; each subject was seen on at least one of these occasions with participation rates falling by only 12% between February and June; for children, the figures relate to June only.

TABLE 2

Number of samples of blood and hair, and of the different environmental samples in Leadhills and Wanlockhead and in Moniaive, the control village.

LEADHILLS/WANLOCKHEAD	Blood	Hair	Garden Soil	House Dust	Hand Wipe	Kitchen Surface Wipe	Domestic Water	Airborne Dust
Men	55	55	15	53	54	54	53	19
Women	71	71	27	70	71	70	70	29
Children	22	21	7	21	19	21	20	9
TOTAL	148	147	49	144	144	145	143	57

MONIAIVE	Blood	Hair	Garden Soil	House Dust	Hand Wipe	Kitchen Surface Wipe	Domestic Water	Airborne Dust
Men	43	43	20	42	41	41	42	9
Women	41	41	10	40	40	40	40	5
Children	15	15	5	14	14	14	15	1
TOTAL	99	99	35	96	95	95	97	15
OVERALL TOTAL	247	246	84	240	239	240	240	72

Note: An overlap of environmental samples exists for persons from the same household: 36% for house dust, kitchen wipes and water; and 43% for soil and airborne dust.

2.3.2 Blood Lead Concentrations

Blood lead results are presented in Table 3 and show consistently higher means for Leadhills/ Wanlockhead compared with Moniaive in men, women and children ($p<0.001$). Children in the old lead mining area exhibited the highest mean blood lead level of 17.6 $\mu g100ml^{-1}$ compared with 10.4 $\mu g100ml^{-1}$ in the children of Moniaive. For men and women, the values recorded were 15.9 $\mu g100ml^{-1}$ Pb and 12.4 $\mu g100ml^{-1}$ Pb in Leadhills and Wanlockhead and 11.0 $\mu g100ml^{-1}$ and 8.3 $\mu g100ml^{-1}$ in Moniaive, respectively. The percentage increases in mean blood lead in Leadhills and Wanlockhead compared with the control were 45% for men, 50% for women and 70% for children (all values measured at $p < 0.001$). Statistically significant differences were evident for blood lead between men and women in both Leadhills and Wanlockhead ($p<0.001$) and in the control population ($p<0.01$), men having the higher lead levels. Children in the former lead mining area had significantly greater blood leads than women ($p<0.001$) but no difference was found with men. No adult/child differences were noted in Moniaive. Blood lead levels are therefore clearly elevated in the contaminated villages when compared with those in a similarly rural setting with no history of lead mining.

2.3.3 Hair Lead Concentrations

The analytical data for hair lead levels is given in Table 4. No marked difference was observed for either men or women between the villages. In the contaminated villages, a mean of 37.7 μgg^{-1} Pb was observed in men and 30.7 μgg^{-1} in women, while in the control area the mean lead measurement for men was 29.9 μgg^{-1} and 28.9 μgg^{-1} for women.

TABLE 3

Mean blood lead levels in the two areas.

	BLOOD LEAD ($\mu g 100ml^{-1}$)					
	Men		Women		Children	
	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)
LEADHILLS/WANLOCKHEAD	55	15.95 (5.39)	71	12.43 (5.18)	22	17.61 (5.39)
MONIAIVE	43	10.98 (4.56)	41	8.29 (3.11)	15	10.36 (3.32)
		***		***		***

Note 1: Student's t-test used to test for significance where $^{***}p<0.001$.
2: Conversion: Traditional to SI units lead, 20.72 $\mu g 100ml^{-1}$ = 1 μmol.

TABLE 4

Geometric mean hair lead levels in the two areas.

	HAIR LEAD ($\mu g g^{-1}$)					
	Men		Women		Children	
	n	Mean (95% Range)	n	Mean (95% Range)	n	Mean (95% Range)
LEADHILLS/WANLOCKHEAD	55	37.7 (10.2 - 138.7)	71	30.7 (10.7 - 88.1)	21	40.2 (14.3 - 112.7)
MONIAIVE	43	29.9 (7.6 - 118.6)	41	28.9 (8.8 - 94.8)	15	12.6 (4.9 - 32.5) ***

Note 1: Log transformation used.
Note 2: Student's t-test used to test for significance where ***$p<0.001$.

For children on the other hand there was a three fold difference ($p<0.001$) between the two areas with means of 40.2 μgg^{-1} Pb in Leadhills and Wanlockhead, and 12.6 μgg^{-1} Pb in Moniaive. Hair lead concentrations were higher in children than adults in Moniaive ($p<0.001$ for both sexes) but not in Leadhills and Wanlockhead. There were no differences between men and women.

2.3.4 Environmental Samples

The results for the lead analysis of five environmental samples are given in Table 5.

Garden Soil

Values for the control village were low when compared with the former lead mining area, with geometric means of 213 μgg^{-1} Pb and 6902 μgg^{-1} Pb, and ranges of 51-887 μgg^{-1} Pb and 1954-24,378 μgg^{-1} Pb, respectively, ($p<0.001$). These results indicate that soils in Leadhills/ Wanlockhead are heavily contaminated.

House Dust

House dust levels showed a five fold increase in mean concentration of lead in the former mining area compared with the control. The geometric mean for households in Leadhills and Wanlockhead was 1570 μgg^{-1} Pb, significantly different ($p<0.001$) from the Moniaive value of 320 μgg^{-1} Pb. The respective ranges of each of these areas were 370-6668 μgg^{-1} Pb and 53-1919 μgg^{-1} Pb and therefore showed some overlap. These values can be put into perspective by referring to the Royal Commission on Environmental Pollution ninth report (1983), which states that the lead concentration of household dust is usually less than 1000 μgg^{-1} Pb.

TABLE 5

Means and corrected ranges for total environmental lead levels in the two areas. Garden soil, house dust, kitchen surface, domestic water and airborne dust lead.

		LEADHILLS/WANLOCKHEAD		MONIAIVE		
		n	Mean (95% Range)	n	Mean (95% Range)	
A	SOIL LEAD ($\mu g g^{-1}$)	28	6902 (1954 - 24,378)	20	213 (51 - 887)	***
B	HOUSE DUST LEAD ($\mu g g^{-1}$)	88	1570 (370 - 6668)	65	320 (53 - 1919)	***
C	KITCHEN SURFACE LEAD ($\mu g m^{-2}$)	89	14.1 (1.5 - 130.6)	64	5.4 (1.2 - 25.0)	***
D	DOMESTIC WATER LEAD ($\mu g l^{-1}$)	89	16 (1 - 70)	66	11 (2 - 30)	**
E	AIRBORNE DUST LEAD ($\mu g^{-1} g^{-1} day^{-1}$ of exposure)	30	2.19 (0.54 - 8.93)	10	0.15 (0.06 - 0.40)	***

Note 1: For A, B, C, and E, the statistical tests were made after log transformation. For D, cube root transformation was used. *** $p<0.001$; ** $p<0.01$.
2: Hand lead levels are shown separately in Table (?).

Hand and Kitchen Surface Wipes

The results for hand wipe lead are shown separately in Table 6 while those for kitchen surface lead are given in Table 5. For men, women and children in Moniaive the values for lead on both hands were low at 8.1, 4.7 and 10.5 μg, with a similar mean concentration of 5.4 μgm^{-2} Pb for kitchen surfaces. However, in Leadhills and Wanlockhead values were significantly higher for both hands and kitchen surface lead compared with the control ($p<0.001$). The amounts of lead on hands were: in men 28.5 μg, in women 12.2 μg and in children 40.8 μg and statistically greater in men than in women in both areas ($p<0.001$ in Leadhills and Wanlockhead and $P<0.05$ in Moniaive). Children from each area had statistically higher levels than women ($p<0.001$), but not for men. For hand lead in men from Leadhills and Wanlockhead the excess amounted to approximately 250% when compared with Moniaive; for women 160% and for children 290%. In both the old lead mining area and in the control, the mean hand wipe levels for women were about half those for men.

The mean value of 14.1 μgm^{-2} for kitchen surface lead in the contaminated area was 160% higher than in Moniaive.

Domestic Water

The data for domestic water are for random day-time samples only, since these are thought to be more representative of a persons intake than first flush water (DOE, Pollution Paper No. 12, 1977; Elwood et al, 1984, and Moore et al, 1977). Mean water levels of lead in the two areas were generally low. However in Leadhills and Wanlockhead the mean value was 16 μgl^{-1} Pb and in Moniaive 11 μgl^{-1} Pb, with the difference significant at $p<0.01$. Mean values for each area fell below the recommended EEC limit of 50 μgl^{-1} (Directive 80/778/EEC).

TABLE 6

Geometric means and corrected ranges for hand lead levels in the two areas (μg per pair of hands).

	Men		Women		Children	
	n	Mean (95% Range)	n	Mean (95% Range)	n	Mean (95% Range)
LEADHILLS/WANLOCKHEAD	54	28.5 (1.5 - 543.3)	71	12.2 (1.5 - 99.8)	19	40.8 (8.6 - 192.7)
MONIAIVE	41	8.1 (0.9 - 73.3)	40	4.7 (1.3 - 17.0)	14	10.5 (5.0 - 22.0)
		***		***		***

Note 1: Student's t-test used to test for significance after log transformation, where ***$p<0.001$.

Airborne Dust

Results for airborne dust lead contamination using the moss bag technique are presented in $\mu g g^{-1} day^{-1}$ of exposure and clearly show a difference between the mean levels of 2.19 in Leadhills and Wanlockhead and 0.15 in Moniaive ($p<0.001$).

Lead In Home Grown Vegetables

Table 7 presents the values for lead concentration in various types of vegetables in the two areas. Mean lead levels in the four types of vegetables, potato (0.14 $\mu g g^{-1}$), cabbage (0.09 $\mu g g^{-1}$), carrot (0.19 $\mu g g^{-1}$) and lettuce (0.21 $\mu g g^{-1}$) from Moniaive, fall well below the limit of 1 $mgKg^{-1}$ (fresh weight) proposed by the Food Additives and Contaminants Committee (FACC) in 1975, which came into operation in 1980 (The Lead in Food Regulations, 1979). Guide-lines prior to this date were that levels should not exceed 2 $mgKg^{-1}$ (DOE, 1982, Pollution Report No. 11).

In Leadhills and Wanlockhead, cabbage and potato mean lead levels were 0.23 and 0.38 $\mu g g^{-1}$, respectively, falling within recent Guide-lines but carrot and lettuce exceeded 1 $mgkg^{-1}$ with an approximate mean of 2.0 $\mu g g^{-1}$. Lead levels in potatoes ($p<0.001$), cabbage ($p<0.05$), carrot ($p<0.01$) and lettuce ($p<0.01$) were significantly higher in the former lead mining villages compared with Moniaive.

Table 8 summarises the data for blood lead levels according to the proportion of home-grown vegetables consumed by the subjects in the two areas.

TABLE 7

Lead in vegetables grown in Leadhills and Wanlockhead, and in the control village, Moniaive (μgg^{-1} fresh weight).

LEADHILLS/WANLOCKHEAD

			Number in each of the following ranges:				
	n	Mean	<= 0.1	0.11 - 0.2	0.21 - 0.5	0.51 - 1.0	> 1.0
Potato	16	0.38	1	5	8	1	1
Cabbage	13	0.23	3	4	5	1	0
Carrot	10	2.15	0	0	0	1	9
Lettuce	7	1.80	1	1	0	0	5

MONIAIVE

			Number in each of the following ranges:				
	n	Mean	<= 0.1	0.11 - 0.2	0.21 - 0.5	0.51 - 1.0	> 1.0
Potato	14	0.14	4	7	3	0	0
Cabbage	7	0.09	4	3	0	0	0
Carrot	11	0.19	1	6	4	0	0
Lettuce	11	0.21	1	6	4	0	0

Note: $1\mu gg^{-1}$ of lead in food (fresh weight) is the current acceptable limit.

TABLE 8

Mean blood lead levels in the two areas in subjects grouped by their consumption of home grown vegetables.

	BLOOD LEAD ($\mu g 100ml^{-1}$)						
	Homegrown Vegetable Consumption						
	None		Some		All/Most		
	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)	
LEADHILLS/WANLOCKHEAD							
Men	23	14.92(4.77)	24	14.71(3.52)	8	22.17(7.87)	***
Women	35	12.43(5.18)	26	11.81(5.59)	10	13.47(4.77)	
Children	10	16.78(4.97)	10	18.86(6.22)	2	14.71(0.21)	
MONIAIVE							
Men	10	12.02(3.52)	20	8.91(3.73)	13	13.05(5.39)	
Women	8	9.95(4.14)	23	7.04(2.07)	10	9.95(2.90)	
Children	5	8.08(2.49)	9	10.98(3.11)	1	15.95 (-)	

Note: Student's t-test used to test for significance, where ***$p<0.001$

A highly significant difference was found ($p<0.001$) in blood leads for men grouped by their consumption of home grown vegetables in the former mining area, between both 'all/most' and 'some' consumption and 'all/most' and no consumption of vegetables. No significant differences were found between groups for women and children in Leadhills/Wanlockhead or for men, women and children in Moniaive. There was no evidence from these results of an overall step-wise increase in blood lead in relation to a greater consumption of home-grown produce.

2.3.5 Seasonal Variation In Lead Contamination

The results for seasonal variation in Leadhills and Wanlockhead for paired samples of men and women from February and June 1984 for blood and hair lead are shown in Table 9. Table 10 looks at seasonal variation in environmental factors over the same period. No results are available for airborne dust lead in February.

When comparisons were made between the two means for February and June using a paired students t-test, blood lead was found to be significantly higher in June than February for men, $p<0.05$, and women, $p<0.001$. Hair lead was also significantly higher in June for both sexes ($p<0.001$), while hand lead showed a similar seasonal trend at $p<0.05$ and $p<0.01$ for men and women, respectively. No significant seasonal variation existed for these variables in Moniaive.

For the paired samples of kitchen surface wipes, water and dust, the paired t-tests showed no significant seasonal variation in lead content. Only women's hand lead ($p<0.05$) and kitchen surface lead ($p<0.05$) were significantly higher in June in Moniaive.

TABLE 9

Seasonal variation in Leadhills and Wanlockhead - paired samples of mean blood and hair lead (sd or 95% range) from February and June.

LEADHILLS AND WANLOCKHEAD

	No of Pairs	February	June	
MEN				
Blood Lead ($\mu g100ml^{-1}$)	40	15.33 (5.59)	16.37 (5.80)	*
Hair Lead (μgg^{-1})	39	25.9 (6.4-105.4)	48.0 (12.9-179.1)	***
WOMEN				
Blood Lead ($\mu g100ml^{-1}$)	67	11.40 (5.39)	12.64 (5.18)	***
Hair Lead (μgg^{-1})	63	22.6 (7.2-71.3)	33.3 (8.2-135.2)	***

Note 1: Log transformation used for hair lead.
2: Paired t-test used to test for significance where $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.
3: None of these variables were measured for the under twelves in February.

TABLE 10

Seasonal variation in Leadhills and Wanlockhead - paired samples of mean environmental lead factors (95% range) from February and June.

LEADHILLS AND WANLOCKHEAD

	No of Pairs	February	June
HOUSE DUST ($\mu g g^{-1}$)	72	1600 (350-7311)	1626 (344-7674)
HANDS ($\mu g pair^{-1}$)			
(a) Men	41	18.8 (1.6-225.4)	36.4 * (1.9-699.8)
(b) Women	65	7.5 (1.1-53.8)	13.3 ** (0.9-202.8)
KITCHEN SURFACE ($\mu g m^{-2}$)	74	12.0 (1.6-87.5)	13.5 (0.9-207.5)
DOMESTIC WATER ($mg l^{-1}$)	32	0.015 (0.001-0.058)	0.017 (0-0.093)

Note 1: Following log transformation, paired t-tests showed no significant seasonal variation in environmental factors, apart from hand lead.

2: $^{*}p<0.05$; $^{**}p<0.01$.

Seasonal variation in blood lead and various environmental factors which might determine blood lead have been recorded in various other studies. The significance of these points will be raised under discussion.

2.3.6 Relationships Between Indices Of Lead Contamination

Simple correlation coefficients were calculated to investigate the relationship between blood lead, the dependent variable and the independent environmental factors: these are shown in Table 11. In the contaminated villages, adult men showed a significant positive correlation between blood lead and the environmental factors of hand lead, kitchen surface lead and water lead (all at $p<0.05$), while women exhibited a highly positive correlation between blood lead and water lead ($p<0.001$). Like the men in Leadhills and Wanlockhead, those in Moniaive also displayed a correlation between blood lead and the environmental factors hand lead and kitchen surface lead ($p<0.01$). Lead in women's blood in Moniaive showed a positive correlation with house dust lead, as well as kitchen surface lead ($p<0.05$). No positive correlations were obtained for children in Leadhills and Wanlockhead, although in the control area a correlation ($p<0.05$) was established between blood and water lead.

In addition, the degree of association between the environmental factors themselves was measured and correlation coefficients for these are given in Table 12. Significant correlations were attained in the two areas between house dust and kitchen surface lead ($p<0.01$) and between airborne dust and kitchen surface lead ($p<0.005$). No significant correlation was achieved between any of the other environmental variables in either area.

TABLE 11

Correlation co-efficients for relationships of blood lead with the various environmental factors in the two areas.

	Garden Soil	House Dust	Hand Wipe	Kitchen Surface Wipe	Airborne Dust	Domestic Water
Leadhills + Wanlockhead						
Men	0.02 (n=15)	0.11 (n=53)	0.28* (n=54)	0.34* (n=54)	0.20 (n=19)	0.34* (n=53)
Women	-0.34 (n=27)	0.06 (n=70)	-0.11 (n=71)	-0.09 (n=70)	-0.02 (n=29)	0.43*** (n=70)
Children	-0.31 (n=7)	-0.02 (n=21)	0.38 (n=19)	-0.01 (n=21)	0.55 (n=9)	-0.14 (n=20)
Moniaive						
Men	0.43 (n=20)	0.30 (n=42)	0.41** (n=41)	0.33** (n=41)	0.65 (n=9)	0.24 (n=42)
Women	0.45 (n=10)	0.33* (n=40)	0.15 (n=40)	0.35* (n=40)	0.40 (n=5)	0.04 (n=40)
Children	0.22 (n=5)	-0.20 (n=14)	0.24 (n=14)	0.39 (n=14)	- (n=1)	0.52* (n=15)

Note: *$p<0.05$; ** $p<0.01$ and ***$p<0.001$.

TABLE 12

Correlation co-efficients between various environmental factors in the two areas.

		Garden Soil	House Dust	Kitchen Surface	Airborne Dust	Domestic Water
Garden Soil	L/W (n=28)	1.00	0.29	0.32	0.30	-0.32
	M (n=20)	1.00	0.23	0.31	0.29	-0.07
House Dust	L/W (n=88)		1.00	0.27**	0.20	0.05
	M (n=65)		1.00	0.37**	0.38	-0.10
Kitchen Surface	L/W (n=89)			1.00	0.44*	0.06
	M (n=64)			1.00	0.72*	-0.09
Airborne Dust	L/W (n=30)				1.00	0.00
	M (n=10)				1.00	-0.01
Domestic Water	L/W (n=88)					1.00
	M (n=69)					1.00

Note 1: **$p<0.01$, *$p<0.05$

2: L/W = Leadhills and Wanlockhead, M = Moniaive.

Further to this, the dependence of blood lead on the levels of lead in the various environmental sources was examined by multiple regression. Two 'categorical variables' were also included as independent variables namely Area and Group, to account for any systematic differences which might exist between the two chosen areas and their populations. A step-wise (forward inclusion) multiple regression was used (Nie et al, 1975), where the order of inclusion, after area and group were inserted into the model, was determined by the contribution of each variable to the variance (Table 13).

The advantage of multiple regression is that it establishes the effect of each independent variable with the other independent variables kept constant. The regression equation is also given in Table 13 where the accumulated percentage of variance explained was 43.6: data transformation improved the fit. A further examination of the data in Leadhills and Wanlockhead alone (n=55) generated the step-wise inclusion regression shown in Table 14 where the accumulated percentage of variance explained was 29.1. The Group effect was dropped in this analysis because of smaller sample numbers, especially for children.

2.3.7 Reproducibility Of Laboratory Estimations

As outlined in the previous chapter, where possible 1 in 10 samples collected for laboratory analysis were run as 'blind' duplicates. The resultant coefficient of variation (SD/mean x 100%) of blood lead estimation based on 75 blind duplicate samples of venous blood was 5%. This figure was obtained from the results achieved by Glasgow Royal Infirmary together with those from six other laboratories involved in the Supraregional Assay Service Lead Scheme.

TABLE 13

Multiple regression analysis of variation in blood lead in the two areas of Leadhills/Wanlockhead and Moniaive (n=71).

Dependent variable: Blood lead
Independent variables: Area, Group, House-dust lead, Hand lead, Kitchen-surface lead, Airborne-dust lead and Domestic-water lead.

Order of Inclusion	Accumulated percentage of variance explained
Area	13.7
Group	23.4
Domestic water	34.4
Hand	39.9
Airborne dust	43.0
Kitchen surface	43.4
House dust	43.6

Regression Equation:

$$\text{Blood Pb} = 1.52\ \sqrt[3]{\text{domestic water Pb}} + 0.15 \log \text{hand Pb} + 0.18 \log \text{airborne dust Pb} + 0.06 \log \text{kitchen surface Pb} + 0.06 \log \text{house dust Pb} + \text{constant}^{*}$$

*Constant values:

Leadhills/Wanlockhead	Men	-0.14
	Women	-0.25
	Children	0.01
Moniaive	Men	-0.05
	Women	-0.16
	Children	0.10

Note: All the data for the environmental levels (hand, airborne dust, kitchen surface and house dust lead) transformed to logarithms. Domestic water lead transformed using the cube root.

TABLE 14

Multiple regression analysis of variation in blood lead in the former lead mining area of Leadhills and Wanlockhead (n=55).

Dependent variable: Blood lead
Independent variables: Hand lead, Kitchen-surface lead, Airborne-dust lead and Domestic-water lead.

Order of Inclusion	Accumulated percentage of variance explained
Domestic water	12.9
Hand	26.5
Airborne dust	28.9
Kitchen surface	29.1

Regression Equation:

$$\text{Blood Pb} = 1.59\ \sqrt[3]{\text{domestic water Pb}} + 0.24 \log \text{hand Pb} + 0.18 \log \text{airborne dust Pb} - 0.03 \log \text{kitchen surface Pb} - 0.03$$

Note 1: All the data for the environmental levels (hand, airborne dust and kitchen surface lead) transformed to logarithms. Domestic water lead transformed using the cube root.

2: Data for men, women and children pooled.

The coefficient of variation for various environmental factors after transformation to logarithms was 9% for garden soil (6 dwellings); 11% for the assorted garden vegetables (15 samples); 22% for house dust samples (57 samples); and 18% for airborne dust lead using the moss bag technique, the latter representing the mean for 9 duplicates run on two separate occasions.

The low value for the coefficient of variation for bloods is impressive and highlights the strict quality control system implemented. The higher coefficient of variation for the environmental samples even after logarithmic transformation was nevertheless within acceptable limits. The variation will partly reflect the greater difficulty of sampling solids. Results for reference materials were always within 10%.

Overall consideration of results from this study indicate that despite great variability there is a general increase in lead exposure in the former lead mining area compared with the control. The significance of this, and other related factors is fully discussed later.

2.4 DISCUSSION

The objectives were to examine the blood lead levels in a random sample of the adult population and all children in the two former lead mining villages of Leadhills and Wanlockhead, and to compare this with a similar population in a control village, Moniaive. In addition, possible sources of exposure to lead were investigated through the determination of lead in environmental variables in the two areas. Taken as a whole the response rates for the study were good; the study population was well defined; and there was a good measure of the environmental exposure of each individual with adequate quality control. These factors provide a firm foundation for the analysis, interpretation and discussion of the

results and the formulation of meaningful conclusions.

The discussion has been organised by first comparing the mean blood and environmental results with those established in previous studies, followed by an examination of the determinants of blood lead. Attention is also given to seasonal variation in lead status.

2.4.1 Blood Lead Levels

Blood lead levels in the inhabitants of Leadhills and Wanlockhead would, like other former lead mining areas, appear to be elevated when compared with other uncontaminated rural areas. Published data for blood lead concentrations in rural areas of the British Isles are scarce and comparisons with the present study are limited. The most relevant comparisons of soil lead and blood lead in the present study and in two other rural studies carried out in past mining areas of the UK for women and children are shown in Table 15. Neither of these studies investigated blood lead levels in men.

The result for blood lead in the rural control area for women in Henllan is very similar to that in Moniaive, while blood lead concentrations for men and women on a remote small island were 8.91 $\mu g100ml^{-1}$ and 7.04 $\mu g100ml^{-1}$, respectively (Elwood and Blaney 1983), slightly lower than the values established in the present control population.

Lead concentrations in blood in Leadhills/Wanlockhead are similar to those reported by the D.O.E. (1981, 1982) for urban areas of Great Britain (Table 16). Further work for the D.O.E. (Quinn, M.J. and Delves H.T., 1987, 1988) confirmed the earlier findings for exposed groups while reporting the blood lead levels of control groups from Birmingham and North Petherton of 10.77 $\mu g100ml^{-1}$ for men and 7.87 $\mu g100ml^{-1}$ for women, respectively.

TABLE 15

Comparisons of soil lead ($\mu g g^{-1}$) and blood lead ($\mu g 100ml^{-1}$) concentrations in the present study and in other similar studies carried out in the UK, for women and children.

	Soil Pb	Number of Children	Number of Women	Mean blood Pb Children	Mean blood Pb Women
Contaminated Areas					
Leadhills/Wanlockhead	1954 - 24,378	22	71	17.61	12.43
Derbyshire*	1050 - 28,000	48	44	24.86	18.44
Halkyn**	140 - 10,181	61	58	22.58	11.81
Control Areas					
Moniaive	51 - 887	15	41	10.36	8.29
Derbyshire*	130 - 3000	34	30	20.93	14.71
Henllan**	21 - 280	32	33	17.61	7.87

Note 1: Figures for soil lead are 95% ranges obtained after log transformation, with the exception of Derbyshire which are minima and maxima.

2: Venous blood was taken from adult women in each survey and from children in Leadhills/Wanlockhead and Moniaive. The other child blood levels represent capillary samples.

3: *Barlop et al, 1975; **Gallacher et al, 1984.

TABLE 16

Mean venous blood lead levels in $\mu g100ml^{-1}$ found in adult men and women in surveys in British cities in 1979-1980 (DOE 1981, 1982).

	Men		Women	
	n	Mean (sd)	n	Mean (sd)
Leadhills + Wanlockhead	55	15.96(5.39)	71	12.43(5.18)
Moniaive	43	10.98(4.56)	41	8.29(3.11)
Inner City Areas				
Birmingham -Handsworth	55	15.13(1.45)	44	11.19(1.66)
-Sparkbrook	46	16.37(1.24)	51	11.81(1.45)
Greater London -Islington	39	14.09(1.45)	48	9.95(1.45)
-Lamberth	95	14.30(1.45)	105	10.57(1.24)
Leeds	55	17.41(1.45)	45	13.68(1.45)
Liverpool	43	16.16(1.24)	57	12.85(1.45)
Manchester	46	19.68(1.24)	54	14.92(1.24)
Sheffield	52	16.37(1.24)	48	13.05(1.24)
Outer City Areas				
Birmingham	46	12.02(1.45)	54	10.36(1.45)
Greater London -Kingston/Thames	122	13.26(1.45)	36	9.32(1.24)
-Walthom Forest	95	11.19(1.45)	102	8.29(1.45)
Leeds	45	15.13(1.24)	56	11.81(1.24)
Liverpool	37	16.16(1.45)	63	11.81(1.45)
Manchester	50	17.61(1.24)	51	15.75(1.45)
Sheffield	47	14.71(1.24)	52	11.81(1.24)
Glasgow (city wide)	98	19.89(1.45)	98	14.71(1.45)

Note: The mean levels in the DOE Surveys are all geometric means.

A recent study of 6-9 year old children in Edinburgh (Fulton et al, 1987) showed a geometric mean blood lead of 9.95 $\mu g100ml^{-1}$ (n=495) which is close to the rural Moniaive child mean. Comparisons with results for other groups of children of a similar age are made in Table 17. As with adults, the high values for children from Leadhills/Wanlockhead are similar to those from other exposed urban populations.

The blood lead results for Leadhills/Wanlockhead can be placed in context with the standards outlined by the EEC (Directive L.229) for populations (Table 18). Despite being close to urban concentrations in this country, it can be seen that all blood lead levels for Leadhills and Wanlockhead and Moniaive fall within EEC recommendations.

A consistent pattern can be seen in terms of the effects of age on blood lead. Throughout the studies cited adults have lower blood lead levels than children (Barltrop et al, 1985; Gallagher et al, 1984). The present study confirm these findings: thus children have 40% higher blood lead levels than women and 10% higher than men in Leadhills and Wanlockhead while comparable figures for children in Moniaive read as 25% and 5%, respectively. A study carried out in Wales further highlights these differences for adults and children (Gallacher et al, 1984): in a former mining area, children had 80% higher blood lead levels compared to their mothers, while the difference for the control area was recorded as 120%.

Differences in blood lead levels according to sex are common in the literature. In the present work blood lead levels in men from Leadhills and Wanlockhead, and from Moniaive are approximately 30% higher than the corresponding women groups. This average figure is equivalent to that found in many other studies which have recorded sex differences for blood lead in adults.

TABLE 17

Mean blood lead levels in children ($\mu g100ml^{-1}$ with s.d.) aged under 12 years of age found in various other studies.

	n	Mean blood lead	
Leadhills/Wanlockhead	22	17.61	(5.39)
Moniaive	15	10.36	(3.32)
Birmingham* (all children)	319	23.42	(12.64)
Newport* (lead workers' children)	51	18.24	(8.91)
Newport* (living near lead works)	170	15.75	(6.16)

Note 1: Blood lead values are for capillary samples with the exception of Leadhills/Wanlockhead and Moniaive, where venous blood was taken.

2: *DOE 1981, 1982.

TABLE 18

Mean blood levels and population distributions in Leadhills and Wanlockhead and in Moniaive compared with the EEC directive (L.229) guidelines.

	Mean blood level	Percentage Over:- 20 μg (0.97 μmol)	30 μg (1.45 μmol)	35 μg (1.70 μmol)	No. of people with values > 20μg
Leadhills/Wanlockhead					
Men	15.96	16.4	1.8	0.0	9
Women	12.43	7.0	1.4	0.0	5
Children	17.61	13.6	4.5	0.0	3
Moniaive					
Men	10.98	2.3	0.0	0.0	1
Women	8.29	0.0	0.0	0.0	0
Children	10.36	0.0	0.0	0.0	0
E C Guideline		<50%	<10%	<2%	

Note 1: Results are in $\mu g 100ml^{-1}$, where 20.72 μg = 1 μmol.

An average figure of 30% higher blood lead in men compared to women was recorded by Mahaffey et al, (1982); Elinder et al, (1983) and in the EEC surveys (DOE, 1981). Due to the small numbers of children in the present study, sex differences were not investigated, although other research would indicate the difference, if any, to be small (Yankel et al, 1977; Raab et al, 1987; Quinn and Delves, 1987; and Baghurst et al, 1992).

The explanations for age and sex differences in blood lead concentrations for populations are likely to be wide ranging, covering behavioural patterns and occupation types. Full consideration will be given to such factors in a later discussion.

2.4.2 Hair Lead

Unlike blood lead, hair lead geometric means show no significant difference for adults between Leadhills/ Wanlockhead and the control area (Table 4). Only children have greater hair lead levels in Leadhills and Wanlockhead compared to those in Moniaive ($p<0.001$). Hair lead values in the former lead mining area are three times greater than those reported in children by Barltrop et al. (1975) in Derbyshire's former mining area where hair samples were washed prior to analysis for lead. Since blood lead concentrations in the Leadhills/Wanlockhead and Derbyshire studies were raised to similar degrees, the difference in hair lead probably reflects surface contamination.

The measurement of lead in hair and its reliability, compared to that of blood as a measure of body burden of lead are in dispute. First there is the question of washing samples, since hair presents an excellent surface for the binding of exogenous materials in the environment. A wide range of cleansing agents including organic solvents, chelating agents, distilled water and

various detergents, have been used alone or in some combination. According to Taylor (1986) none of these washing procedures completely remove all lead and there is no useful purpose in attempting to cleanse samples. This point is backed by Chattopadhyay et al. (1977).

Secondly there is no standard collection procedure for hair, despite the fact that it is known that lead concentration tends to be highest in hair furthest from the scalp (Cherry, 1981; Barry, 1975 and Hopps, 1977). Hair samples in the present study were taken from the 4 cm nearest to the scalp at the nape of the neck on the assumption these would reflect body lead levels over a 3-4 month period, with hair growing about 1 cm per month.

Discrepancies are also noted in terms of sex effects on hair lead concentrations. For example, conflicting papers show higher concentrations in samples taken from men (Creason et al, 1975; Sky-Peck and Joseph, 1983); no difference for men and women (Weiss et al, 1972; Reeves et al, (1975) and higher concentrations in females (Klevay, 1973; Barry, 1975). In Leadhills and Wanlockhead, and in Moniaive no significant differences between males and females were found. Such differences may depend on the frequency with which men and women wash their hair in the different populations.

Age effects are also disputed. Some authors (Klevay, 1973; Weiss et al, 1972) indicate that hair lead levels in children are about twice those of adults, while others (Creason et al. 1975, Reeves et al., 1975 and Niculesui 1983) reported no change in lead concentration with age. Data for Leadhills/Wanlockhead show no difference between adults and children whereas in Moniaive child lead levels in hair are around half those established in their parents ($p<0.001$). The effect of age may be masked by external contamination of hair in 'high lead' environments.

Of ultimate importance in the assessment of hair lead is the existence of a relationship between hair lead and blood lead levels. Here too, existing information varies. While some authors have found significant correlations between blood and hair (Wibowo et al, 1980; Barltrop et al, 1975), others have not (Barry, 1975; Rockway, 1984; and Ahmed et al, 1989). In the present work inconsistent correlations of blood and hair lead are evident. For Leadhills and Wanlockhead data, no correlation was found for men, women or children. In Moniaive, strong positive correlations of 0.50 ($p<0.001$) and 0.48 ($p<0.001$) were established for men and women, respectively, although no correlation was found for children in this area. This latest research confirms the doubts on the usefulness of lead in unwashed hair as a measure of body lead status.

An analysis of the relationships between hair lead and the environmental factors of soil, house dust, hand and airborne dust lead provided no significant correlations in either area or population grouping. It is therefore questionable whether lead levels in hair not washed in the laboratory provides a reliable measure of environmental burden.

From the present work and other recent research it is clear that hair lead research to date is unreliable. It is therefore recommended that future work is centred on hair samples which are unlikely to receive external contamination such as armpit or pubic hair, a subject which has received only scant examination. Only blood lead levels will be discussed further in this paper as being the best indicator of body lead burden.

2.4.3 Environmental Sources Of Lead

Garden Soil

Garden soils from Leadhills and Wanlockhead are heavily

contaminated with lead, and with a geometric mean of 6902 μgg^{-1} were until recently the highest occurring mean levels recorded in the British Isles. Moniaive soil lead levels are approximately thirty times lower than in the contaminated area (Table 5).

There are two points which must be considered when making comparisons with published data. The first is the lack of a standard practice for taking soil samples. Some authors sample soil 0-15 cm in depth (Gallacher et al, 1984; Davies, 1983 and Davies et al, 1979) while others use a 0-5 cm sample depth (Angle and McIntire, 1982; Davies et al, 1987 and Davies and Thornton, 1987). Some others have chosen a depth of between 10 and 20 cm (Berrow et al, 1987), although it has long been established that soil lead decreases quite markedly with depth. The second point relates to the presentation of results. Soil lead data normally have very wide ranges which are either given as absolute ranges (Culbard et al, 1983), or as the 95% range, where the distribution is normalised by a suitable transformation (Gallacher et al, 1983).

Comparisons can be made for the present work with a sampling depth of 0-15 cm with the former lead mining village area in Wales (Gallacher et al, 1984) where values were much lower (Table 15). Elwood et al. (1985) found a geometric mean of 180 μgg^{-1} lead in Cardiff soils, while a median soil lead of 436 μgg^{-1} was recorded by Culbard et al. (1983) in the UK 'ten town' survey using the 0-15 cm depth. Work using a 0-5 cm depth in a former Derbyshire lead mining area gave a similar range to Leadhills and Wanlockhead (Barltrop et al, 1975) but had a considerably lower geometric mean of 4881 μgg^{-1}. A more recent study of a Derbyshire village closely associated with historic lead mining gave a soil lead mean of 7140 μgg^{-1} (2400 - 22,800 μgg^{-1}) at a 0-5 cm depth (Cotter-Howells and Thornton, 1991), which is very close to the 0-15 cm sample results in the present mining area. Urban

soil lead values of 404 μgg^{-1} have been found at a 0-5 cm depth in Brighton and Hove (Davies and Thornton, 1987), while the Birmingham reconnaissance study gave a soil lead value of 278 μgg^{-1} (Davies et al, 1987).

There are little comparable data for soils in uncontaminated rural communities. 'Normal' lead values of <110 μgg^{-1} (Davies, 1983) and 5-25 μgg^{-1} (Harrison and Laxen, 1981) indicate mild lead contamination in the mean value of 213 μgg^{-1} in Moniaive. Garden lead concentrations at Moniaive are similar to those expected from suburban gardens as outlined above. Using Moniaive as a reference may slightly underestimate the degree of contamination at Leadhills and Wanlockhead.

Attention has been drawn in this discussion of soil lead values to differences in sampling protocol, although there must be many reasons why differences arise in soil lead values between former lead mining areas. These include how extensive the industry was; how long ago it ceased; attempts to control pollution; the situation of the village in relation to past mining activity; and climatic effects. Nevertheless a common strategy for sampling and presentation of results is recommended so that comparisons between published data are made simpler. Here, a sample depth of 0-15 cm is recommended since it is more likely to reflect the largely adult population's exposure through gardening activities than the 0-5 cm depth. For presentation of results, absolute ranges can give undue emphasis to extreme values and the normalisation method is to be preferred. Some authors have presented their data in both forms (Davies and Thornton, 1987; Davies and Ballinger, 1990).

House Dust

Lead in house dust samples from Leadhills and Wanlockhead, with a geometric mean of 1570 μgg^{-1}, are some

five times higher than those recorded in Moniaive (Table 5).

Like lead in soils, uncertainties arise in comparisons of published data for house dust lead because of differences in terms of collection of samples, the size fraction of dust used for analysis and the presentation of data. Arguably of most importance are the methods used for the collection of dust in homes and the subsequent presentation of results. Some authors (Elwood et al, 1984; Thornton et al, 1985; and Strehlow and Barltrop, 1987) have taken a dust sample from the vacuum cleaner bag and expressed the results in amount of lead per unit weight of dust, i.e. a measure of lead concentration. On the other hand some have used a special vacuum sampling head and thimble over a defined area (Laxen et al, 1988; Davies et al, 1987) with results expressed as amount of lead per unit area. Less commonly employed, Baker et al. (1977) used a wiping technique and Harrison (1979) a sweeping method, with results given as concentrations.

Comparisons for the present study can be drawn from work in the former Welsh lead mining area where Davies et al. (1985) found no striking contrast between sample and control areas with geometric lead means of 346 μgg^{-1} and 169 μgg^{-1}, respectively, observed. Although both studies used similar collection procedures and analytical methods it is evident that Leadhills and Wanlockhead have markedly higher results. Using similar methods to those above, Strehlow and Barltrop (1987) reported house dust lead levels over a three year period from rural Suffolk and central London: Suffolk gave a geometric mean lead level very close to that reported for Moniaive and London a value around half that of Leadhills and Wanlockhead. The Leadhills/Wanlockhead value exceeded the geometric mean for house dust from the towns of Brighton and Hove (Culbard et al, 1988) by 30%. Culbard et al. (1983) also reported a median lead value of 637 μgg^{-1} in house dust

from ten British towns. More recently, a national survey in cities, towns and villages in England, Scotland and Wales established house dust geometric mean lead levels of 507 μgg^{-1} in 3955 samples from outside London, and 1010 μgg^{-1} in 683 London samples. Excluding data from geochemical hotspots, the mean lead concentration was 561 μgg^{-1} overall (Culbard et al, 1988). Given the similarity of sampling techniques and presentation of results in all the above studies, it can be seen that Leadhills and Wanlockhead are consistently high in terms of lead in house dust samples.

Lead concentrations in house dust from the high soil lead area in Derbyshire where dustfalls filters were used for collection (Barltrop et al, 1974) have a geometric mean close to the concentration recorded in Leadhills and Wanlockhead (Barltrop et al, 1975). However house dust levels in Barltrop et al's 'control' area were approximately two and three times greater than in the present and Welsh studies, respectively, with a geometric mean of 565 μgg^{-1} lead.

The lead loading in house dust method, using a vacuum sampling head over a given area, has given the following results. In an inner city study of Birmingham the geometric mean lead loading found in children's bedrooms and playrooms was 62 μgm^{-2}; the geometric mean for these same areas in terms of dust lead concentration was 430 μgg^{-1} (Davies et al, 1987). Other geometric mean lead loadings include a range of 30 to 81 μgm^{-2} in 166 homes from four areas in the Netherlands (Brunekreef et al, 1983); a median lead loading of 680 μgm^{-2} for 12 homes in Champaign-Urbana, USA (Solomon and Hartford, 1977); and a mean lead loading of 636 μgm^{-2} (median 418) for 65 homes in Christchurch, New Zealand (Fergusson and Schroeder, 1985). Differences in actual sampling method applied in such studies leads to difficulty in their interpretation, however the use of an 'excess' measure over the 'rural'

control within studies would allow 'total' figures to be compared with concentrations.

It has been argued that the exposure of children to lead in dust can be expressed more realistically by lead loading than simply lead concentration (Culbard et al, 1986; Davies et al, 1987; and Raab and Fulton, 1987). For example, Davies et al. (1987) found children's blood lead to have a higher correlation with lead loading in house dust (r=0.46) than with dust lead concentrations (r=0.21). On the basis of such work some recent studies have adopted the loading technique (for example, Cotter-Howells and Thornton, 1991). However, Raab et al. (1987) found a slightly lower proportion of the variance in blood lead of children explained by lead loading than by lead concentration in dust. Moreover, a study of a former lead mining area in Colorado, USA found dust loading did not offer any distinct advantage over the concentration measure and applied a μgg^{-1} measure since this corresponded to the units used in other environmental measurements and allowed further modelling (Bornschein et al, 1988). To date there is no definitive evidence on whether a loading or concentration technique should be adopted.

In the present work on house dust analysis, the principal aim was to estimate the variability of dust lead over a few months since this is the time interval over which blood lead responds to changes in exposure. The average age of dust in household vacuum cleaners was found to be between three and four months. Only one home in the present study was found to be without a vacuum cleaner. The fact that there was no seasonal variation in house dust lead concentrations whereas another measure of household contamination (hand lead) did show variation (Table 10) probably reflects the long period over which vacuum dust collected.

The most commonly used size fraction for lead analysis in house dust, including the present work, is 1000 μm (Davies et al, 1990). There have however been suggestions that much smaller particles should be analysed because of their greater contribution to lead intake (Duggan et al, 1985). As with other indices of contamination, presentation and other variations in procedures may mask the overall effect geographical factors present. There is still clearly a requirement for overall standard practices to be agreed on all fronts.

Hand And Kitchen Wipes

The results from the present study are compared in Table 19 with a similar study by Gallacher et al. (1984) in Wales, covering two areas with differing degrees of environmental lead contamination. Hand lead values are three and four times greater, respectively, for women and children in Leadhills and Wanlockhead compared to Moniaive. The Welsh study gives only a one and a half fold excess for lead on hands of women and children in Halkyn, compared to those in their control population.

Hand lead measurements for men in Leadhills and Wanlockhead and in Moniaive are approximately double those for women in each area, with men from the contaminated village having more than three times the level of hand lead compared to the control village (Table 6). Overall, children in the present study populations have the highest concentration of lead on hands. Hand lead levels were not measured in men in the Welsh study. A much greater distinction between 'high' and 'low' lead areas was observed for kitchen surface lead in the present work than in Wales.

TABLE 19

Mean lead levels (μg) on the wet wipes and ranges (95% range) after log transformation - a comparison of the results found in the present study with those found in a similar study in Wales (Gallacher et al,1984).

	Woman's Hands		Child's Hands		Kitchen Surface	
	n	Mean (95% range)	n	Mean (95% range)	n	Mean (95% range)
Contaminated Areas						
Leadhills/Wanlockhead	71	12.2 (2-100)	19	40.8 (9-193)	89	14.1 (2-131)
Halkyn	36	13.2 (3-67)	36	20.4 (5-87)	36	13.5 (3-69)
Control Areas						
Moniaive	40	4.7 (1-17)	14	10.5 (5-22)	64	5.4 (1-25)
Henllan	24	9.6 (2-53)	24	14.1 (6-32)	24	10.0 (2-42)

Studies of hand lead contamination tend to follow one of two methods: a paper towel soaked in an alcohol-based cleansing solution (Present Study; Gallacher et al, 1984; Sayre et al, 1974; Davies et al, 1987) or the rinsing of hands with a dilute acid solution (Roels et al, 1980; Brunekreef et al, 1987). A further difference is that some assess lead on both hands (e.g. Sayre et al, 1974) while others make their measurement from the 'dominant' hand only (e.g. Brunekreef et al, 1987).

Davies et al. (1987) measured lead on both hands of 2-year-old children from an inner city area of Birmingham using a wipe method. Hands were wiped using three wet wipes over eight consecutive days giving a geometric mean value of 5.7 μg. In the Welsh study (Gallacher et al, 1984), 1-3 year olds in the high lead area had a mean more than three times the Birmingham value. Using a similar wipe technique Duggan et al. (1985) found means ranging from 12-43 μg Pb for hands in eleven infant schools in London.

Results for children in a rural school were broadly compatible to those in Moniaive while urban values ranging from 12.7 to 20.4 μg were generally much lower than those in Leadhills and Wanlockhead where a range of 8.6 to 192.7 was observed (Roels et al, 1980). Hand lead levels found by rinsing the dominant hand in 4-6 year old Dutch children from inner city areas had a mean value of 12 μg (Brunekreef et al. 1983).

The effect of method applied on the end result for hand lead measurements has not been studied directly but the values quoted above for cities do not indicate a major difference between 'wipe' and rinse methods. The present data confirm that age and sex can influence the end result and should be accounted for when making population comparisons. It should also be borne in mind that given levels of contamination for children and adults may not

represent common levels of lead ingestion. There must be a sizeable difference between children and adults in the frequency with which unwashed hands enter the mouth or touch food. It is unknown how much lead is removed by rinsing hands during normal life.

For kitchen surface lead, there is a requirement to measure the area of the food preparation surface wiped. In the Welsh study (Gallacher et al, 1984) no area is given for comparison with the present work. Further differences will arise due to other factors such as the length of time dust accumulates on a given surface since the last routine kitchen cleaning was done.

If the relationship between hand and kitchen surface lead and for example, blood lead is to be accurately determined and comparisons made between studies, then the above are questions which should be answered by future study. With the methods chosen for the present study, neither hand nor kitchen wipe lead was consistently correlated with blood lead within villages and population groups (Table 11). In terms of 'excess' both methods tended to overestimate the lead contamination in Leadhills/Wanlockhead when compared with blood lead data.

As a final point, it is noted that most authors concentrate on the implications of lead on children's hands without consideration of the implications for adults. The present results suggest that adult hands may be an important pathway whereby lead enters the body and is given full attention in the integrating discussion.

Domestic Water

The mean household water value in Leadhills and Wanlockhead is 16 $\mu g l^{-1}$ and in Moniaive 11 $\mu g l^{-1}$ (Table 5). These values differ, proportionately, by as much as blood lead between the two areas. The two communities receive

water from different areas and different reservoirs. However, the Leadhills water source is situated on the outskirts of the geological lead area with the possibility of a mineral source affecting the water supply. This may explain the slightly higher levels found in the former lead mining villages compared to those in Moniaive. There were few incidences of lead piping in homes in both areas.

Thomas et al. (1979) stated that there was no evidence that any of the water sampling methods used - day-time, running and first flush - was a markedly better predictor of a persons lead intake than the others. On the other hand, D.O.E. (1977) and Moore et al. (1977) preferred the use of a random day-time sample. First flush samples have been found to give a low correlation with blood lead (Elwood et al, 1976; Moore et al, 1977), while flushing of a tap before sampling the water can significantly reduce the value. (Thomas et al, 1981; Wong and Berrne, 1976). More recently, the Edinburgh lead study used a 30 minute stagnation sample, (Raab et al, 1987) a method backed by Bailey and Russell, (1981). There is also clearly a requirement for further research on this front in order that specifications might be made on which method is to be preferred, as well as providing upper limits for lead in water.

There is little likelihood that the method chosen in the present study, a random day-time sample, affects the conclusion that both communities consumed water minimally contaminated by lead according to current legislation. Of more concern is the fact that while the water at both locations had lead concentrations within the limits found in many surveys (Gallacher et al, 1984; Thomas et al, 1979), and are well within the European Community Directive 80/778/EEC of 50 μgl^{-1}, both simple and multiple regression analysis indicated a significant effect of intake of lead from drinking water on blood lead. This

will be discussed again later.

Airborne Dust

Several studies have employed the technique used in the present work to monitor airborne dust lead. A geometric mean figure for lead in airborne dust of 2.19 $\mu gg^{-1}day^{-1}$ and 0.15 $\mu gg^{-1}day^{-1}$ was found in Leadhills/Wanlockhead, and in Moniaive, respectively, using the moss bag method (Table 5). Allowing for probable differences in design, the geometric mean figures for the present study are in broad agreement with those obtained by Davies and White (1981) and with Gailey and Lloyd (1986), where mean lead concentration in moss bags was reported as 6.10 $\mu gg^{-1}day^{-1}$ and 5.01 $\mu gg^{-1}day^{-1}$, respectively, in contaminated areas, and 0.60 $\mu gg^{-1}day^{-1}$ and 0.14 $\mu gg^{-1}day^{-1}$, respectively, for background areas. Similar techniques employed by Little and Martin (1974) over a large area around a zinc and lead smelting complex near Bristol indicated much higher levels of environmental contamination than in Leadhills/Wanlockhead.

The moss bag technique may not give a quantitative assessment of air lead contamination and results should not be compared with data obtained by conventional air monitoring methods. Nevertheless this simple method is valuable in examining relationships and patterns of atmospheric contamination over a wide area, where availability of conventional equipment is limited (Gailey and Lloyd, 1986).

It is interesting that the measurement of lead in airborne dust in Leadhills/Wanlockhead gave a particularly large excess over Moniaive compared with most other measures of environmental contamination. Furthermore, some correlations with blood lead were the largest obtained although the small number of samples precluded a significant link.

Like many other techniques, further investigation and standardisation, for example of the type of moss employed, is required to achieve uniformity of results, although the shape of the moss bag (spherical, flat, etc.) will depend on the source(s) and distribution of metals. Whatever the technique, it is most important that 'air' lead has been measured in a way relevant to the actual exposure of individuals. While indoor lead measurements may be preferred on the basis that an individual spends most time indoors, all measurements whether in or out of doors should be made at a height and situation relevant to exposure as in the present work.

Garden Vegetables

Fresh weight vegetable lead levels in Leadhills and Wanlockhead, ranging from 0.23 μgg^{-1} in cabbage, 0.38 μgg^{-1} in potatoes, to approximately 2.0 μgg^{-1} in carrot and lettuce (Table 7), can be compared with similar classes of vegetables from Shipham. There, vegetables were also prepared ready for eating or cooking prior to analysis using normal household methods of washing, peeling, etcetera. Summer crops of Shipham cabbage and carrot had means lead levels of 0.46 μgg^{-1} and 0.49 μgg^{-1}, while lettuce had a value of 0.30 μgg^{-1} and potatoes 0.16 μgg^{-1}, overall being between two and six times lower in lead for the latter three vegetables when compared to the present samples (Sherlock et al, 1983). Values for the same vegetables grown in a market garden environment were all below 0.1 μgg^{-1} lead, suggesting very mild contamination of samples in the present control village, where levels ranged from 0.09 μgg^{-1} in cabbage to 0.21 μgg^{-1} in lettuce (MAFF, 1982).

It is generally held that although the lead content of plants reflects the amount of lead in soils, a significant increase in the lead content of soil results in only a small increase in plant lead concentration and

that only a small proportion of lead in soil is available for plant uptake (Davies and Thornton, 1988). This situation arises from chelation of lead in the root cell walls which effectively produces a barrier and restricts the movement of lead to the above ground parts of plants (Zimdahl, 1976; Davies and White, 1981).

Dispute still however exists concerning how much lead in plants has arisen from surface / aerial contamination, which may not easily be removed on washing. In the present old lead mining area there is a clear possibility of aerial contamination of vegetables through lead dust blown from the spoil heaps or from 'soil splash'. It was established that a 'normal' wash procedure likely to be implemented by a house owner in Leadhills/Wanlockhead using tap water removed between 40 and 65 percent of externally deposited lead from the leafy surfaces. Published data for the removal of external sources of lead from crops varies quite dramatically (Royal Commission of Environmental Pollution, 1983; de Temmerman and Vandermeiren, 1987). Aerial deposition of lead further adds to the soil lead pool and is then available for plant uptake, the vertical distribution of metals in soil lending another complicating factor to the picture.

It has been shown that a number of variables besides soil and airborne lead can play an important part in determining the concentration of lead in plants. Soil variables such as pH, phosphorus supply, organic matter, moisture status, texture, as well as the presence of other heavy metals in soil can affect plant uptake of lead. For example, it is known that plant uptake decreases with increasing soil phosphorus, organic matter content and pH (Khan, 1980; Purves, 1985).

From results for lead in vegetables grown in Leadhills/Wanlockhead and Moniaive (Table 7) it is evident that in Leadhills and Wanlockhead carrot and

lettuce have higher concentrations of lead than cabbage and potato. Results from Wales for these four species lists carrot and lettuce as having elevated concentrations of lead (Gallacher, 1985, Personal communication). Similar collection and preparatory techniques were used in both these studies prior to analysis. A number of the points outlined earlier on factors influencing lead concentrations in vegetables may be applied to explain this since Welsh soils were significantly lower in lead than those in the present old lead mining area.

These apparent interactions between site and species affecting vegetable lead concentrations may be further attributable to local factors such as foliar contamination and preparation procedures carried out by the housewife. Differences in the physical structure of foliage must play a significant part in capturing aerial lead in different vegetables species. For example, a more 'open' growing lettuce must trap more aerial lead than a 'closed' cabbage. Additional determinants of surface contamination include ground cover, rainfall, wind and the length of time plants have been exposed. It is known through rigorous wash and extraction tests (Little, 1973) that some lead on plant species may be incorporated into the cuticle and cell walls of leaves.

Coarse leaves have been found to contain up to ten times more lead than younger leaves. Other work highlights the potential for uneven distribution of lead in root vegetables with lead generally found to concentrate in skins or rinds (MAFF, 1972; Davies and Thornton, 1988). The removal of skins or outer leaves of vegetables in each study introduces the possibility of operator bias. While each operator may be consistent in his/her technique within a study they will almost certainly vary in the proportion of the vegetable which they discard. The proportion discarded may also be influenced by the

variety grown and its maturity when harvested.

Vegetables at sites were collected in the summer period: this is important since lead uptake varies seasonally. During periods of active growth a decrease in concentration may be expected, while much higher herbage lead levels found in autumn and winter are thought to be a result of redistribution of lead previously bound within the root. Clearly plants with different root systems will encounter different concentrations of lead, since the surface horizons of most soils have higher concentrations than the lower horizons.

The contribution of the lead contaminated vegetables at Leadhills and Wanlockhead to body lead burdens will of course depend on consumption patterns. The effects of eating 'none', 'some' or 'all/most' of such vegetables grown in a contaminated and uncontaminated area are addressed in a following discussion.

2.4.4 Seasonal Variability

The seasonal variability in data collected for blood and hair in men and women and in various environmental variables of exposure from Leadhills and Wanlockhead are shown in Table 9 and 10, respectively. The fact that blood lead levels are higher in June than in February for adult men and women ($p<0.05$ and $p<0.001$) is not unique. Other studies which found a higher level of lead in summer include Billick et al. (1979) in a study of paediatric blood lead levels in New York, a report by the World Health Organisation (1977) and Barltrop (1979) who found higher blood lead levels in summer, compared to those in spring. However, EEC blood lead surveys in 1979 and 1981 found very little variation from month to month for adults or children, while Delves et al. (1985) reported no marked seasonality in serial blood lead concentrations in UK adults. No seasonal variability in

blood lead for adults in Moniaive was established.

In an attempt to explain differences in the literature, the above study areas were classified contaminated with lead or not. However, seasonality effects in blood lead are independent of type of area. For example, the contaminated areas of New York and Leadhills showed summer peaks while the EEC surveys (made up of adults and children subjected to environmental lead through living near lead works, roads and cities, or with plumb solvent water supplies) showed no seasonal variation. Barltrop (1979) found summer lead levels elevated in mothers and children in both contaminated and control towns in Derbyshire compared to those in spring. An examination of age and sex in these studies yielded no further explanation for the inconsistencies.

Where seasonal variation in blood exists there is some doubt concerning to what degree. In the present work an increase of 6.8% for men and 10.9% for women is evident in Leadhills/Wanlockhead in June compared to February. These figures can be compared with overall seasonal variability in the New York paediatric population examined by Billick et al. (1979) where a change of 8% was found and in cord blood samples (i.e. neonates) examined by Rabinowitz and Needleman (1982) who described seasonal changes of 15%. Overall, these and the present figures for Leadhills and Wanlockhead are not overwhelmingly different, but studies do differ in population age, highlighting the requirement to compare like with like.

Seasonal variation of lead concentration in hair does not seem to have been well studied, possibly because of the inherent difficulties experienced in using this material as an indicator of body lead. Nonetheless highly significant differences ($p<0.001$) are present in men and women from Leadhills and Wanlockhead in accordance with

season, with hair lead levels elevated by 85% and 47%, respectively, in summer. No differences were noted in Moniaive. Hand wipe levels of lead also exhibited seasonal variation in the former lead mining area, being significantly higher in June for men ($p<0.05$) and even more significantly for women ($p<0.01$) when compared to levels in the February collection of data. Furthermore, the seasonal increases in hair and hand wipe lead were of similar magnitude, suggesting that they reflected a common source of contamination such as airborne lead. Only women in Moniaive had significantly higher summer hand lead ($p<0.05$).

Table 10 shows that kitchen surface lead, house dust lead and water lead in the former lead mining area remained constant over the seasonal sampling period. House dust levels and the question of seasonal variation do not seem to have been well studied. The current data however is in accordance with Laxen et al. (1988) and Strehlow and Barltrop (1987) who reported no significant seasonal effect in dust lead concentration. The vacuum bag method is not ideal for detecting seasonal changes since values represent the dust which has accumulated over the preceding 3-4 months. Lead in water can vary as a function of temperature and hence of season. Lacey (1985) for example has provided data from the Water Research Council indicating water lead levels can double in the temperature range 5°C to 25°C. No seasonal change was evident for water, which is perhaps not unusual considering the smaller range in temperature experienced between these months in the remote upland location of Leadhills and Wanlockhead. No seasonal variation was noted for water and house dust in Moniaive, although kitchen surface lead was greater in the summer ($p<0.005$).

The seasonal variation in Leadhills and Wanlockhead for samples of blood, hair and hand lead but not house lead,

indicates that the main source of body lead is likely to be the outdoor environment. Greater seasonality in blood and hand lead in women may reflect the longer number of days/time spent out doors in the summer months involved in such occupations as gardening. Such time spent outdoors, could feasibly result in lead contaminating hands, hair and ultimately blood levels. The existence of such pathways are considered later.

The present use of moss bags as a measure of monthly airborne lead dust gave varying results according to climatic conditions throughout the nine month study. For example, it is known that being ectohydric, sphagnum moss can absorb cations from rainwater with metal uptake greatly maximised when moss is wet (Gailey and Lloyd, 1986). Wind speeds also affect the collection rate of moss bags, since high winds dilute the concentrations of metal particles in air, but also cause a greater volume of air to filter through. How far the lead concentrations found in the present moss bags were influenced by weather is unclear. However with data unavailable for December, January and February, any obvious increase in lead concentration in the wettest months may have been missed. What is clear is that the moss bag technique is not a good measure of seasonal variation in exposure to lead. Further research on airborne dust lead using moss bags should investigate and select the most reliable months for associations with blood lead. A higher winter rainfall for example may provide evidence of inverse seasonality.

Overall, it would appear that the question of seasonal variability in lead data is not well understood. How great an effect this might have on annual lead exposure is uncertain, and may be a subject worthy of investigation.

2.4.5 Determinants Of Blood Lead

In order to investigate the relationship between blood lead levels and the independent environmental variables, simple correlation co-efficients were calculated as shown in Table 11. To add to the picture, the relationships existing between the environmental variables are also presented in Table 12. The difference between village means in blood lead and a particular environmental variable indicate probable routes for lead to enter the body. The widespread variation in blood lead present within each population could be explained by local variation in exposure and shown by significant correlations with the same environmental variables.

Garden Soil

No significant correlations exist between blood lead and garden soil lead for men, women or children in Leadhills and Wanlockhead, or in the control area of Moniaive. The present results agree with those of Barltrop et al. (1975) in their study of an old lead mining area in Derbyshire. Lead in soil is probably a relatively minor source of exposure. This is not unexpected considering how rarely a person might be in contact directly with soil and the degree of personal hygiene implemented. It was noted that a lot of gardens were grassed over particularly in Leadhills and Wanlockhead with gardening curtailed through poor climate and environmental conditions to small allotment areas. A far greater amount of gardening and cultivation was evident in Moniaive which may explain the positive, though not significant, correlations between garden soil and blood lead in Moniaive adults. It is likely that only those adults heavily involved in gardening or children engaged in play activities might be affected by lead in contaminated garden soil.

However, several authors have shown a positive correlation between blood lead and soil lead in young children using an 0-5 cm soil depth sample. For example, Yankel et al. (1977) established a significant relationship between the blood of one to nine year old children and composite surface soils contaminated by a lead smelter in Northern Idaho, while a seven year study in Omaho in three defined areas (Angle and McIntire, 1979) also gave a positive correlation for blood and soil lead in one to five year olds ($p<0.01$).

A shallow soil sample probably best reflects the soil lead exposure associated with the play activities of the young children sampled. It may be that consideration should be given to depth of soil sample according to age groupings of the population since a 0-15 cm depth of soil is probably more representative of soil lead exposure in adults engaged in gardening activities. Surface soil lead may, however, make a significant contribution to airborne dust and household lead exposure in adults and children.

Duggan and Inskip (1985), and Chaney and Mielke (1986) indicate from a literature review that, on average, blood lead levels increase by 5 $\mu g100ml^{-1}$ for a 1000 μgg^{-1} rise in soil lead. Blood lead levels in both adults and children from Leadhills and Wanlockhead are appreciably lower than might have been expected from this relationship. This may be a reflection of the differences in chemical form in which lead is found in different locations and its resultant bioavailability. For example, the main lead source in Leadhills and Wanlockhead is lead sulphide (galena), which is distinctly less soluble than lead contamination from smelters (Yankel et al, 1977) paint, fossil fuels or car exhausts. Blood lead levels established in a former lead mining village in Derbyshire also fail to meet the above prediction (Cotter-Howells and Thornton, 1991). Despite a similar soil lead mean and range being found to that in Leadhills and Wanlockhead,

blood lead levels in children are much lower in Derbyshire. It has been suggested that the lower blood leads in Derbyshire may be attributed to the chemical form of lead being pyromorphite which is less soluble than galena.

From these studies it is evident that soil lead solubility should also be measured when determining relationships between soil lead and blood lead. Records of time spent out of doors and behavioural factors may improve soil/blood lead relationships.

House Dust

Another possible source of lead entry to the body may be through house dust lead, part of which may be contributed by soil particles from footwear, household pets, and wind blown soil. Nevertheless, the results of correlation analysis between blood lead and house dust lead show no significant relationship for men, women or children in Leadhills and Wanlockhead, while in Moniaive a significant positive relationship was present only in women ($p<0.05$), although it approached significance in men ($p<0.15$). The absence of a consistent relationship between blood and dust lead in each area is in accordance with information presented by Yankel et al. (1977) in their study near a lead smelter in Northern Idaho. There, dust samples obtained from vacuum cleaner bags did not significantly correlate with blood lead concentrations, though it is pointed out that half the homes in this study had no vacuum cleaner or samples were unavailable. In Barltrop et al's (1975) study of 'high' and 'low' soil areas in Derbyshire no significant correlation between lead in house dust and blood was found.

Other studies have, however, reported a significant positive correlation between blood and house dust lead. For, example, in Omaha, Angle and McIntire (1979)

reported a significant relationship for blood lead of pre-school ($p<0.001$) and 6-18 year old children ($p<0.001$) and house dust lead collected from vacuum cleaners. Many other studies have pointed to house dust lead as a factor influencing blood lead levels (for example, Vostal et al, 1974; Charney, 1982; Brunekreef et al, 1987) although there is little direct evidence of such a pathway. Landrigan et al. (1975) for example, found a very significant relationship between blood lead and dust lead in an area with a mean of 4022 $\mu g g^{-1}$ dust lead, but not in areas with means of 922 and 816 $\mu g g^{-1}$ dust lead.

The contribution of soil lead to dust lead may be determined from correlations between environmental variables in Table 12. In Leadhills and Wanlockhead only 8% of the variability in dust lead can be explained by soil lead, while in Moniaive the figure is even less at 5%. These figures are lower than those from a Welsh lead mining area and a control area, where 27% of the variability in dust lead was explained by soil lead (Davies et al, 1985). Davies and Thornton (1987) provided a similar positive correlation ($p<0.001$) with 30% of the variability in dust lead being explained by soil lead in the two towns of Brighton and Hove. A highly significant correlation ($r=0.56$, $p<0.001$) has also been reported between the two variables by Culbard et al (1983, 1986). Angle and McIntire (1979) showed a positive correlation for the two variables in a 'high' lead area, but interestingly the figure of 7% for the contribution of soil lead to variation in dust lead was similar to that in Leadhills and Wanlockhead. Like the latter area, Barltrop et al. (1975) found there was no significant correlation between the two concentrations within a given area, this result being further backed by the work of Moorcroft et al. (1982).

There are many other factors which may affect the lead content of house dust and hence the above findings.

According to Inskip and Hutton (1987) these include:

1. the condition of the paint work i.e. sound or flaking;

2. the presence or absence of lead based paint on indoor and outdoor surfaces;

3. the recent history of home renovation work;

4. the presence of atmospheric lead sources such as refineries and smelters;

5. the traffic density of the neighbourhood;

6. the state of home cleanliness and cleaning regimes exercised;

7. the lead content of garden soil;

8. others, such as occupationally related intake of lead dust into the dwelling or position within the home where the samples are taken.

Of these, refineries and smelters can be ruled out for Leadhills/Wanlockhead and Moniaive, while the traffic density of the neighbourhoods was judged to be similarly light at less than 500 vehicles per day. The presence or absence of interior lead paint was not investigated since other research (Department of the Environment 1981) has shown that it is now unusual for lead in paint to be found indoors in this country. On the other hand, it has been found that blood lead levels tend to correlate with the age of housing property (Department of the Environment, 1981; Culbard et al, 1983). Many studies have shown a correlation between blood lead levels and age of dwelling, especially those built before 1945 where older paints are likely to have been used (Department of the Environment, 1981). Both the 'lead' and control areas in the present work have property of around 150 years

old. Other authors (Duggan and Inskip, 1985; Inskip and Atterbury, 1983; Inskip and Hutton, 1987; and Laxen et al, 1987) have also pointed to the significance even today of raising house dust levels of lead by stripping old layers of paint and redecorating.

Differences exist between soil lead levels and house dust lead levels within each of the two areas. In Leadhills and Wanlockhead house dust levels are approximately four times lower than those in soils, suggesting a dilution effect from intrinsic sources such as carpet fluff and some of the factors mentioned above. On the other hand house dust lead levels are around one and a half times greater than levels in soils from the control area. This would suggest a considerable contribution of lead from sources within the home, adding to those from the exterior environment.

The overall absence of consistent relationships between blood lead and house dust lead in the present study areas suggest that house dust levels of lead were only slightly related to the individuals lead exposure in a direct way but there is much conflicting information on this front. More recent studies (Duggan et al, 1985; Davies et al, 1987) point to the importance of dust lead as a source of blood lead, but suggest the greater importance of hand lead as the pathway for dust lead to enter the body. The significance of a dust-hand-mouth pathway from the present results are discussed below.

Hands

An important route for environmental lead ingestion is through what has been termed the hand to mouth pathway, i.e. the transfer of environmental lead on hands to the mouth and into the body by a variety of mechanisms. Such mechanisms might include for example, eating food with dirty hands or placing the hand in the mouth on a regular

basis, which many children have a tendency to do. A number of studies have attempted to examine the relationship between hand lead and blood lead levels. For example, Charney et al. (1980) established a significant relationship between the two parameters in children, while Gallacher et al. (1984) found hand lead to be a major predictor of blood lead in children ($r=0.38$; $p<0.05$) but not in women in an old lead mining area.

Further evidence arises from work by Sherlock (1987) in an investigation of lead in food and the diet, where it was found that children who washed their hands before eating meals or snacks tended to have the lowest blood lead concentrations: Strehlow and Barltrop (1987) reported a similar tendency.

Hand wipe levels of lead in the present study indicate a large excess in the 'contaminated' area which might well contribute to the elevated blood lead levels. This is supported by the correlation between hand and blood lead (Table 11). There is an indication that men are more susceptible to the hand transfer of lead than women : this is a probable reflection of differences in day to day activities and the quality of personal hygiene. The positive correlation between hand and blood lead for children in Leadhills and Wanlockhead ($r=0.38$) approached significance ($p<0.15$) despite the small sample size. Furthermore correlations are evident not only in the 'high' lead area but also in men in the control village. The failure to establish an association of significance for blood lead and hand lead levels for women in Moniaive may also be attributable in part to a higher degree of personal cleanliness.

There has been much discussion about lead in dust as the main source of lead on hands, commonly known as the dust-hands-mouth route. One of the first to show evidence for this phenomenon was Sayre et al. (1974) who found a clear

relationship between lead on individual hand and in household dust (from floors, window-sills and walls) for children aged between one and six years. Duggan et al. (1985) showed a significant relationship between the hand lead of five and six year old children and dust lead levels measured in school playgrounds. Lead in playground dust and lead on hands was significantly related in children aged nine to fourteen years living in the vicinity of a Belgian primary lead smelter (Roels et al, 1980). Research by Davies (1990) provides more recent evidence of a link between the two factors in a study of two year old children in Birmingham. Here, a positive correlation of 0.21 between lead concentration in floor dust from the children's bedrooms and playrooms and hand lead was established, although a correlation of greater magnitude was found (0.46) between dust lead loading (i.e. results expressed as amount of lead per unit area) and hand lead. Bornschein et al. (1988) also proposed a pathway between floor dust lead and hand lead in six year old children from the former site of an extensive lead mining and milling operation.

While hand dust lead is assumed to be of considerable importance as a body lead source, especially in children, little reliable quantitative information is available (Davies and Thornton, 1988). However, the results for correlation analysis in the present study emphasise the importance of house dust as a source of lead transferred by the hands. In the control village of Moniaive, significant positive correlations were established between the two factors of 0.35 ($p<0.05$) in men and 0.44 ($p<0.01$) in women. Like Moniaive, there was a strong positive correlation between house dust and hand lead in women ($r=0.33$, $p<0.01$) from Leadhills and Wanlockhead, although not in men. The poor correlation for men from this area may reflect a lesser time spent in the domestic environment. The lack of significant correlations in data for children in each area may be due to the small sample

size : since a relationship is evident in adults, then there is likely to be a quantitatively more important route in children.

When correlation analysis was undertaken to indicate the extent to which garden soils could be a source of lead on hands entering the body, no relationship was identified (on average r=0.09) in any area or age group. This result upholds the earlier comments that soil lead appears to be relatively insignificant as far as blood lead levels are concerned in this study. It may be that stable isotopes could be used in the future to determine more accurately what percentage of hand lead may have come from the garden.

Research to date on the hands to mouth route has centred on the importance of this route for children largely because there are some basic differences between adults and children in lifestyle and susceptibility to lead exposure. Young children are generally the highest risk group and tend to show higher blood lead levels than adults (Baltrop et al, 1975; Gallacher et al, 1984) and the present study is no exception (Table 15).

There are however some interesting contrasts between studies. The child : women ratio for blood lead in the contaminated areas of Leadhills and Wanlockhead and Halkyn (Table 15) are similar whereas child hand lead levels in the former area show a three fold excess over adult women, and only a one and a half excess in Halkyn (Table 19). It may well be that the much younger children studied in Halkyn (1-3 years) had their hands 'wiped' regularly by their parents, compared to the older child population in Leadhills/Wanlockhead. Decontamination through mothering activity may also explain the low hand lead levels reported by Davies et al (1990).

Mouthing activity in young children is probably a most important factor influencing the amount of lead absorbed

into the body. Sucking objects contaminated by house dust, playing on the floor and touching household surfaces or eating food contaminated by hand and/or household surface lead could all contribute to the burden of lead in the body.

The child populations in Leadhills and Wanlockhead and Moniaive were relatively old, but a significant pathway may nevertheless exist for lead to enter the system through hand lead contamination. Because of small sample numbers the effects of age or sex of the children on hand lead were not examined. It is however likely that child sex differences for hand lead might be evident in the older age groupings, with boys showing higher concentrations as a reflection of their chosen play activities (Yankel et al, 1977; Roels et al, 1980). In the present adult male population in particular, hand lead is important as a predictor of blood lead, although straightforward hand to mouth activity is unlikely. This is of considerable significance given that most studies dismiss the prospect of hand-mouth transferral of lead being of importance other than for children.

The method of using wet wipes for the determination of lead on hands (and household surfaces) might appear somewhat crude. It is recognised that wet wipe levels measured from any source must vary greatly throughout the day and with the activities of individual personnel. Despite this, Davies et al. (1987) found little variation in daily levels of lead on children's hands, while Gallacher et al. (1984) showed the method of wet wipes to be surprisingly reproducible with a coefficient of variation of around 19%.

Kitchen Surfaces

In the present work correlation data for food preparation surfaces show similar associations with blood lead levels

in each area to hand lead. There were significant positive relationships between kitchen surface and blood leads (Table 11) for men in Leadhills and Wanlockhead ($r=0.34$, $p<0.05$) and for men and women in Moniaive ($r=0.33$, $p<0.01$; $r=0.35$, $p<0.05$, respectively). Furthermore, house dust lead and airborne dust lead both show significant positive correlations with kitchen surface lead ($r=0.27$ with $p<0.01$ and $r=0.44$ with $p<0.05$, respectively, in Leadhills and Wanlockhead; $r=0.37$ and $r=0.72$, respectively, in Moniaive with the same levels of statistical significance - Table 12). These results would suggest that a pathway exists for lead moving into the food chain through contamination of kitchen surfaces.

Correlations between hand wipes and kitchen wipes for adults in the former mining area gave r values of 0.32 ($p<0.05$) and 0.11 for men and women, respectively, while in Moniaive the r values were 0.17 and 0.58 ($p<0.01$) for men and women. In Leadhills and Wanlockhead, stronger correlations for men were perhaps surprisingly also found between kitchen wipes and blood lead. Because they spend more time outdoors, men would be expected to have higher hand lead and hence blood lead contamination from the exterior environment. Since household lead in Leadhills and Wanlockhead comes predominantly from external sources and the house is probably a 'protected' and less contaminated environment, contamination of the house and of women may be given undue emphasis in lead mining areas. In Moniaive men and women showed similar correlations for lead in blood and kitchen wipes but women showed by far the strongest correlation for hand and kitchen wipes depicting their time spent in the kitchen.

The data for lead removed by wet wipes intimate that in areas of 'high' and 'low' environmental lead to greater and lesser degrees, a reduction in blood lead concentration could well arise from improved standards of

personal and domestic hygiene. This supports earlier work which investigated the local environment and related blood lead levels to the cleanliness of the home and maternal involvement with the child. For example, Charney et al. (1983) lowered blood lead levels of children by introducing wet mopping programmes to homes and by giving parents instruction on the washing of their child's hands. While Dietrich et al. (1985) supports this general view of the importance of home environment and maternal care, research by Bellinger et al. (1986) tends to disagree. Blood lead levels of children in the latter study were, however, quite low and perhaps reflect their parent type who were well educated and socially advantaged. Socially advantaged groups and lead contaminated areas are not usually found together. The Ernhart and Morrow-Tlucak (1987) study in Cleveland, U.S.A. of disadvantaged children emphasised the importance of social variables and the quality of maternal care.

The present study establishes the wet wipe technique in both child and adult populations as a means of detecting the pathways of lead into the body. Dust lead is probably available on all domestic surfaces and can therefore act as an extensive source of body lead. Davies et al. (1990) and Harvey et al. (unpublished) have pointed to the importance of portable video cameras in assessing a child's normal behaviour in touching objects in their domestic setting, and hence the intensity of object-hand-mouth activity. The present study results suggest that this procedure could be usefully extended to measure behaviour and hand to mouth contamination in older age groups.

It is recommended that further research on this front be refined with measurements made of contaminant particle size to make a complete assessment of how much lead from dust is ingested and subsequently absorbed into the

system.

Domestic Water

It has long been established that a relationship exists between high water lead levels and elevated blood lead levels but the context has usually been that of contamination from lead pipes. For example, Thomas et al. (1979) studied blood lead levels in mothers and young children in two housing estates, one receiving their water supply through lead pipes, the other through pipes replaced by copper. Water lead levels were markedly higher in the 'lead estate' when compared to the 'copper estate' and blood lead levels in mothers and children from the former area more than double those in the latter. The replacement of lead pipes reduced water lead levels in the 'lead estate', and after 6 months blood levels in both estates were indistinguishable. Moore (1977) provided similar data for the effect of lead piping on blood lead levels for water supplies in Glasgow.

Sherlock et al. (1982) considered both diet and drinking water as sources of lead exposure for mothers and children living in Ayr; they established a positive relationship between increased blood lead and increased drinking water lead. Yet another example was provided by Pocock et al. (1983) whose study of middle aged men from British towns showed blood lead levels to almost double when comparing the lowest to highest water lead levels.

As well as removal of lead pipes other steps can be taken to reduce water and subsequently blood lead levels. For example, in two of the above areas pH levels of the water were raised to reduce plumbsolvency, resulting in a drop in water lead concentration of 80% (Sherlock et al, 1983) and a clear fall in blood lead levels in adult women (Moore et al, 1981).

In many instances therefore, water has only been regarded as an important contributor to body lead in areas with a plumbsolvent water supply. In areas where lead in water supply meets or is below the EEC recommendation of 50 μgl^{-1} (EEC Community Directive 80/778/EEC) lead intake from water is very often disregarded. For example, Roels et al. (1980) examined blood lead levels in children attending schools near a smelter and dismissed water as a source of lead on the basis that in the four areas under study, drinking water did not contain more than 50 μgl^{-1} Pb. Angle and McIntire (1979) also dismissed water Pb in an environmental lead and children study in Omaho. Workers may have been misled by studies which have found no significant correlations between blood and water lead due to water sampling techniques which wrongly estimated actual consumer intake, a point which was discussed earlier.

The positive correlations between lead concentrations in blood and drinking water found in the present study, where mean water lead levels are at low and 'acceptable' concentrations of 16 μgl^{-1} in Leadhills and Wanlockhead and 11 μgl^{-1} in Moniaive, provide an important contrast to such literature. The results in Table 11 for correlation of blood and water in the former lead mining area gave a significant co-efficient for men ($r=0.34$, $p<0.05$) and a highly significant co-efficient for women ($r=0.43$, $p<0.001$), perhaps reflecting the fact that women spend more time in the home environment. The significant positive correlation for children in Moniaive ($p<0.05$) should be viewed with some caution when the small sample number is taken into account. The results are consistent with a report that reducing lead in water from 10-19 μgl^{-1} to <10 μgl^{-1} results in a significant fall in blood lead concentrations (Sherlock et al, 1984).

Many authors have chosen to use a cube root model between water lead and its relationship with blood lead, since

the latter has been found to vary more closely with the transformed data (Sherlock et al, 1982; Moore et al, 1979; Elwood et al, 1984). At very low water lead contentrations the cube root method has been held to over exaggerate the relationship. However, similar values to those in the present work have been successfully transformed using the cube root in other studies (Elwood et al, 1984). Other models, for example, logarithmic and linear have also been employed, leading to difficulties in making comparisons between studies.

On an individual basis the importance of water lead to body lead uptake will clearly vary. For example, it is known in Great Britain that when average water lead levels rise above 100 $\mu g l^{-1}$, then water lead exceeds food lead as a main contributor to blood lead (for example, Thomas et al, 1979). Today, mains water is generally low in lead, with high levels usually attributed to domestic lead plumbing and storage utilities. The effect of lead piping was found to be particularly marked in a shepherd from the Moniaive population. In the February sample the man had a mean blood lead level of 17.9 $\mu g 100ml^{-1}$ rising to 36.6 $\mu g 100ml^{-1}$ in June, with domestic water lead constant at <10 $\mu g l^{-1}$ throughout the period. After extensive enquiry, the additional source of lead was traced to an outhouse on a hill side which the man had been using for a refreshing long drink of water each day over March, April and May. Extensive lead plumbing and piping was discovered with water lead concentrations ranging between 4300 and 6000 $\mu g l^{-1}$. Being atypical of the control area, results for this gentleman were excluded from the analyses!

The apparent effects of small inputs of lead in the present study (Table 11) on blood lead may reflect the fact that soluble lead is fairly easily absorbed by the body (Heard et al, 1983). Assuming an average water intake of 1.5 litres per day the present results suggest

an intake of 168 μg and 116 μg lead per week from water, for individuals living in Leadhills and Wanlockhead, and in Moniaive, respectively. Both values fall within the 3 mg lead per week currently recommended as acceptable by FAO/WHO. However, if all the lead (mostly insoluble?) recovered from the hands of men, women and children in the two villages were transferred to the body, the total weekly intakes would be 200, 85 and 286 μg, respectively, in Leadhills/Wanlockhead and 57, 33 and 74 μg, respectively, in Moniaive (from Table 6).

The influence of water lead will however depend on additional factors other than concentrations. The availability of lead can be substantially lower in hard water than in soft water areas (Thomas et al, 1981). Very soft water is present in the domestic supplies of Leadhills and Wanlockhead in particular, and also in Moniaive probably enhancing the availability of lead and its transfer to blood. Varying amounts of water are consumed by individuals. Matthew (1981) estimated the relative intake of water in babies being bottle fed to be up to seven and half times greater compared to adults. This effect could be amplified if mothers use hot tap water in the preparation of bottled milk and thus solubilize more lead. The proportionate absorption of lead from water, milk, diluted fruit juices etcetera is likely to be greater than from tea the major drink for many adults in which lead may be precipitated before drinking. It is likely that water intake will vary not only between age groups, but also between sexes, geographical regions and socio-economic groupings.

Water lead levels have been found to be important in the preparation and cooking of foodstuffs which have a tendency to take up lead from water (Little et al, 1981). For example, Smart et al. (1981) found leafy vegetables to take up three times the amount of lead compared to root vegetables such as potatoes. This point is of

relevance to the high lead concentrations established in vegetables in the present work. The uptake of lead during cooking may however, be less if the vegetables are already high in lead : indeed there may even be a net movement from the vegetable if dry matter cellular breakdown occurs.

It has been suggested that protein denaturation during cooking may leave a binding site available for lead attachment. A further study by Smart et al. (1983) highlights the increased uptake by foodstuffs such as rice and spaghetti. Interestingly, foods which gain or lose water during cooking can both increase in lead concentration, so dispelling the thought that simple water absorption increases the food lead concentration but there was little difference in lead uptake from hard and soft waters. These findings show water lead to be influential in another light, and while relevant to blood lead levels, may add to the issue of establishing a relationship between water and blood lead in different populations. Smart et al. (1981) concluded that at average water levels of 20 μgl^{-1} the contribution of water to total dietary intake of lead, taking vegetable uptake into account, is about 10%. Bois et al. (1989) having estimated amount of lead absorbed into the body and applied clearance concept models to the assessment of exposure to lead in drinking water from published works, estimated that the contribution for adults in the general population would be 7% if drinking water contained 10 μgl^{-1} of lead. Using their study it is further evident that drinking water would contribute 22% of blood lead at a 50 μgl^{-1} maximum contaminant level. This research however assumes the unlikely event of a steady state of lead absorbance and lead elimination by the body.

Airborne Dust

Of relevance to all environmental variables mentioned to

date is the contribution made by airborne lead dust. Much research has been conducted in the U.K. to determine lead in air, and in general the concentration of lead in air in rural areas is low (Cawse, 1974; and RCEP, 1983). The use of the moss bag technique in the present work gave geometric mean concentrations of 2.19 and 0.15 $\mu g g^{-1}$ lead day^{-1} in Leadhills/Wanlockhead, and in Moniaive, respectively, indicating that the air in the former area is contaminated with lead dust to a degree comparable with a contaminated area of Wales (Davies and White, 1981). The significant difference in airborne dust lead between areas ($p<0.001$) must be attributed to past mining and the dispersal by wind of dust and debris from spoil heaps. Furthermore airborne dust probably contributes lead to all the environmental variables so far measured including growing crops.

The relationship between air lead and blood lead is not clear with some authors reporting a linear relationship (Angle et al, 1984; Chamberlain, 1983) while most agree that inhalation of air lead is likely to make only a small contribution to body burden of lead (for example, Davies, 1987). The absorption of airborne lead will depend on the size and shape of the particles, the rate of inhalation and the bioavailability of lead in the inhaled particles. Young active children will inhale air in greater amounts and more deeply than older, less active adults and are therefore likely to acquire more lead from air. Some lead may be deposited in the nasal passages and/or swallowed.

The absence of significant correlation coefficients for airborne dust and blood lead in both areas (Table 11) may reflect deficiencies of the moss bag method, the small sample numbers, or discontinuity in time between observations on air and those on blood. Poor relationships between lead in airborne dust and blood cannot simply be explained by the fact that a person

spends the majority of hours in a day in an interior setting. The mean external air lead concentration is a good predictor of the mean internal air lead concentration (Davies et al, 1987). Furthermore the mean of individual internal:external ratios was 0.61 in this particular study based in Birmingham, using conventional air monitoring equipment. Other studies in the UK have indicated a similar ratio (for example, Chamberlain et al, 1978; Elwood, 1983). Thus the lesser time spent outdoors is offset by a higher level of exposure, something which may partially explain the higher blood lead levels in men than in women.

There is evidence from correlation analysis of a relationship between the lead in airborne dust and that found on kitchen food preparation surfaces which may affect the entry of lead into the body. Table12 gives a significant positive correlation between the two factors of 0.44 ($p<0.05$) and 0.72 ($p<0.05$) in Leadhills/Wanlockhead and in Moniaive, respectively. Positive correlations, though not statistically significant, are also evident for airborne dust with house dust lead. From these results it is probable that airborne lead is falling out of suspension and contributing to lead on household surfaces and probably onto food itself. In view of this and given the importance of lead transfer from dust via hands into the body, it is clear that remedial action should be directed against the atmospheric dispersal of lead particulates in Leadhills and Wanlockhead.

Garden Vegetables

A highly significant difference was observed ($p<0.001$) in blood leads for male subjects grouped by their consumption of home grown vegetables in Leadhills and Wanlockhead but not for women or children (Table 8). It could be that men in the former mining area show an

effect due to contact with heavily contaminated soil at all stages of cultivation. A similar analysis by Gallacher et al. (1984) in an old lead mining area in Wales showed significant differences between groups of women who ate different amounts of home grown vegetables. When compared with those who ate 'none' there was a 15% increase in blood leads of those who ate 'some' and a 28% increase in those who ate 'most' vegetables. Like the present study there was no difference in blood leads according to vegetable consumption in the control area of Gallacher et al's (1984) study (blood lead levels in men were not considered). The conclusion by these authors was that the consumption of locally grown vegetables had only a small effect on blood lead, raising the population mean by about 5%. Since most lead concentrations in the contaminated Welsh vegetables were only slightly lower than those in Leadhills and Wanlockhead (Gallacher, 1985, personal communication) the vegetable to blood pathway for lead transport should be of little more importance in the current study.

The study in Shipham (Sherlock et al, 1983) of a former mining area which implemented similar preparatory techniques to the present work indicated that the lead intake of residents, which included home-grown crops of vegetables and fruit, was slightly lower than the national average and crops did not contain excess lead. Wijn et al. (1983), having determined that vegetables grown near busy motorways had between two and three times the lead content of vegetables grown away from trafficked areas, concluded that there was no elevation in the blood lead of those people who ate a large amount of the contaminated vegetables with raised lead levels.

It is likely from the studies in Wales, Shipham, and Leadhills and Wanlockhead that when comparing differences in blood leads according to the amount of home grown vegetables consumed, the degree to which the vegetables

have been contaminated by lead, should be taken into account.

From the present work it is probable that all sexes and ages in Leadhills and Wanlockhead can consume home grown vegetables without further elevating blood lead levels, at the levels of domestic hygiene currently implemented. Only one harvest per annum is possible in Leadhills and Wanlockhead due to climatic factors, limiting contact with vegetable and/or soil lead. This too may limit the effect of lead contamination of vegetables on blood lead levels in the area. The degree of personal hygiene implemented by men during cultivation and harvesting of vegetables could also play an important part in determining blood lead levels.

2.4.6 Multiple Regression Analysis

Since the environmental data suggested multiple pathways whereby lead enters blood, a multiple regression analysis was undertaken in an attempt to isolate the effects of various environmental factors in the two areas. The results for Leadhills and Wanlockhead, and Moniaive are presented in Table 13, along with the regression equation which predicts the mean blood lead levels of the populations. The multiple regression method assumes that the variables are measured without error, but in any case the reproducibility of results was excellent in this study. Furthermore, most environmental factors were correlated with each other and the differentiation between effects will therefore be incomplete. In addition, the results from such an exercise can be sensitive to the type of transformation applied to each data set. Nevertheless the multiple regression model was adopted in view of its wide use in the literature. The model took into account 'Area' differences and also 'Group' differences (the latter including variation in age and sex within areas) which explained 13.7% and 9.7%,

respectively, of variance in blood lead.

This exercise indicated that water lead had the largest influence on blood lead concentrations, 'explaining' 11% of the variance, despite the 'acceptable' low lead levels in water. Furthermore, lead concentrations in drinking water were not correlated with the other environmental sources of lead and are unlikely to have been confounded with them. Hand lead was the next most important factor explaining around 6% of variance; airborne dust lead contributed 3%; while kitchen surface lead and house dust lead explained less than 1% of the variance. Due to the small number of samples, garden soil results were not included in the regression analysis. However from the results for correlations between soil and blood, it is unlikely that soil lead would contribute much to variance in blood lead.

A further examination of the data in the former lead mining area of Leadhills and Wanlockhead alone (Table 14) showed water lead again to be the best predictor, explaining around 13% of variance in blood lead. Thus the contribution of water lead was not dependent on its association with area. Interestingly, hand lead explained 14% of variance in blood Pb in Leadhills and Wanlockhead, more than double the variance accounted for in the two area analysis. The contribution of airborne dust lead was approximately the same at just over 3%, while kitchen surface lead explained less than 1%. House dust lead no longer contributed to this model, which explained 29% of the variability in blood lead overall as opposed to 43.6% with the combined data. Clearly the introduction of 'area' and 'group' factors in the first regression removes part of the effect of some of the environmental variables in Leadhills and Wanlockhead. Because of the relatively small sample size, no separate regression analysis was undertaken for Moniaive.

The amount of variance in blood lead explained by multiple regression can be compared with other studies. Elwood et al. (1984) for example 'explained' 38% of the variance in blood lead in women from five areas of Wales chosen to represent different exposures to traffic, through regression analysis of environmental lead sources of water, air and dust (log blood Pb = 1.06 + 0.18 log mean air Pb - 0.02 log dust Pb + 0.62 water Pb $^{1/3}$). Angle and McIntire (1979) in their study of 6-18 year old boys and girls in three areas (Urban-commercial; in the vicinity of a small battery plant urban-mixed; and a residential/suburban area) of Omaha, U.S.A. found three environmental variables of air, soil and house dust lead to account for 21% of the variance found in blood lead, but because of the multicollinearity of the independent variables, it was not statistically valid to attribute a discrete percentage to each source. In another study the 'best' regression model relating the blood lead of two year old urban children in Birmingham to various sources of lead was : log blood Pb = 0.55 + 0.10 log X1 + 0.14 log PbW + 0.07S, where X1 is the dust lead loading multiplied by the rate for hands touching all objects, PbW is the water lead concentration, and S is a dichotomous variable dependent on whether parents smoked. Overall 35% of the variance in blood lead was explained using this model (Davies et al, 1990). Thus, the present model was more successful than those above in predicting blood lead concentration. Differences however, will clearly arise in comparisons of multiple regression models according to whether studies use data from lead contaminated, non-contaminated or 'mixed' populations and whether 'area' and 'group' effects are considered. Furthermore, the source and hence potential absorption of lead could be important : it may be that urban lead (mainly tetraethyl?) is more available for absorption by man than more insoluble mine waste lead. For example, water lead in Leadhills/Wanlockhead (mean : 16 $\mu g l^{-1}$) is a

more important predictor of blood lead than the highly contaminated environment, yet lower than the geometric mean water lead concentration of 19 μgl^{-1} in Birmingham where much lower levels of environmental lead exist.

There are a number of reasons why a higher percentage of variance in blood lead has not been explained in this and other studies. These may include the exposure of subjects to sources of lead other than those measured. Food, for example may be an important source of lead intake. Whilst lead is generally low in most foods today, it may have been elevated in canned foods, in livers and kidneys, and in some vegetables and herbs. In addition, Sherlock (1987) points to differences in absorption by man depending upon types of food eaten. Individual differences in usage of cigarettes, consumption of alcohol and employment circumstances are other factors which may influence blood lead levels.

However, the major reasons for the incompleteness of model prediction may have been precision errors in sampling the components of the model. The single measures of hand and kitchen surface wipe lead will not measure accurately the amount of lead transferred to the body from hands or contaminated food even in the home environment because of the variable extent to which individuals decontaminate themselves (e.g. by washing or showering). The amounts of lead measured at the time of the study may not have been representative of the mean long term intake which contributed to the more stable blood lead concentration.

The most important conclusion to draw from this multiple regression relates to the major influence of water lead on blood lead, despite the water lead concentrations being well within the E.E.C. limit of 50 μgl^{-1} (Directive 80/778/EEC) and the overwhelming differences in lead concentrations in other environmental sources. The

current results for effects of water lead on blood lead epitomize the importance of complying with the new World Health Organization guideline for lead in drinking water which recommends a change to 10 $\mu g l^{-1}$ in 1993 (W.H.O. 1992, personal communication). A pathway of 'hand lead to body lead' is also strongly suggested particularly in the former lead mining area. It is also interesting to note that airborne dust lead measured by the moss bag technique and contributing around 3% to body lead, equates with other works using conventional air monitoring equipment (for example, Davies et al, 1987).

The overall implications and recommendations of this work are discussed in the final section.

3. LAMB STUDIES

3.1 DETAILED OBJECTIVES

An examination of blood and milk lead concentrations in ewes and detailed blood lead concentrations in lambs was made over the period when clinical symptoms of the locomotor disorder normally develop. In addition, other blood and tissue elements, and soil and herbage elements which may contribute to the problem were investigated. A comparison was made with a similar set of samples from a control farm.

3.2 METHODOLOGY

3.2.1 Study Area

Co-operation for this part of the study was sought from local farmers near the former lead mining villages of Wanlockhead and Leadhills. Consultations led to the identification of a farm on upland moorland in Wanlockhead which experiences losses of between 20 and 40 percent in the lamb population each year from the locomotor disorder described earlier. A farm near Moniaive in an otherwise similar location was chosen for the purposes of comparison. Both farms use Scottish Blackface breeding stock.

3.2.2 Experiment 1

One week prior to the earliest expected lambing date in April 1984, 34 ewes in Wanlockhead and 20 in Moniaive were chosen for closeness of expected lambing. These ewes were tagged and numbered and subsequently sampled to determine blood levels of lead, zinc, copper, calcium and phosphorus.

Of these ewes, 21 in Wanlockhead, and 13 on the control farm produced 24 and 18 lambs, respectively, within a few days of each other, giving two lamb populations of a similar age group for further study. These lambs were tagged and blood sampled at 5-7 days, 4 weeks, 8 weeks and 12 weeks of age for the elements outlined above. By 12 weeks, twelve lambs in Wanlockhead but none in Moniaive, had died. Sample numbers for this study were determined after consulting other studies (Butler et al, 1957; Stewart and Allcroft, 1956), and statistical sources (Armitage and Berry, 1971, 1987).

Investigational Techniques And Specimens

Blood

Both ewes and lambs had wool cut from the neck using stainless steel scissors, and the vein area cleaned using a dilute alcohol solution. Blood was taken using a sterile disposable vacutainer needle (Becton-Dickson) into a 10 ml Vacutainer sterile blood tube containing lithium heparin as an anticoagulant (Figure 10). A separate tube, with a zinc free bung was taken for the determination of zinc levels. Blood samples were transported to the laboratory for analysis the same day.

Lead

From each whole blood sample, two 2 ml aliquots were placed separately in two glass digestion vials using a calibrated pipette. Standard additions of 0.1 and 0.2 $\mu g ml^{-1}$ Pb prepared from BDH stock lead standard solution were added, one to each vial. An acid digestion was then carried out on a heating block using 5 ml of an nitric/perchloric acid digestion mix (4:1). Each sample was taken to dryness and the resulting deposit taken up in 5 ml 6N hydrochloric acid.

Figure 10. Lamb Blood Sampling.

Standards were prepared in a similar fashion in the range 0.1 to 0.5 $\mu g ml^{-1}$ Pb in 5% hydrochloric acid and assayed with all samples on a Il 151 Atomic Absorption Spectrophotometer at 217 nm. From this a calibration graph was plotted for the calculation of results.

Copper

The remaining blood sample tube was spun at 2200 G for 20 minutes on a Multex centrifuge. The plasma was then removed and stored at -15°C prior to analysis. After thawing, 0.42 ml of plasma was pipetted into contamination-free autoanalyser cups. 1.75 ml of 6% Analar butanol was added to each sample to give a 1:5 dilution. Standards were prepared from BDH stock copper standard solution to contain 0.2, 0.6, 1.0, 1.4 and 1.8 $\mu g ml^{-1}$, similarly diluted with 6% butanol. Samples and standards were assayed by aspiration on an Atomic Absorption spectrophotometer (213.9 nm) as outlined above for lead.

Zinc

Zinc concentrations in plasma were determined at wavelength 324.7 nm using the method outlined for copper plasma analysis, substituting zinc standards of the same concentration.

Phosphorus

Plasma phosphorus determinations were made using the Pierce Phosphorus Auto/Stat kit which contains a set of reagents and standards for performing the quantitive determination of phosphorus in serum. 0.1 ml plasma was added to 3.0 ml working reagent in a test tube and mixed well. All test tubes were then allowed to stand at room temperature for 30 minutes and the resulting blue colour

read on a Vitatron colorimeter at 690 nm. The working reagent was prepared by mixing 10 volumes of Reductant Reagent (sodium acetate trihydrate, 10 gl^{-1}; acetic acid, 26 gl^{-1}; sodium metabisulphite, 10 gl^{-1}; p-methylammoniumphenol sulphate, 5 gl^{-1}; also contains a surfactant) with 1 volume of Molybdate Reagent (ammonium paramolybdate heptahydrate, 10 gl^{-1}; sulphuric acid, 21.2 gl^{-1}; also contains a catalyst). The phosphate reacts with molybdate in the acid medium to form the blue phosphomolybdate complex. The two standards 0.97 and 1.62 $mmoll^{-1}$ underwent the same treatment and analysis as serum samples to form a calibration graph for calculation of the results. All absorbencies were read at 690 nm after setting absorbence=0 with the Reagent Blank.

Calcium

0.1 ml of plasma and standards in the range 0.75 to 3.75 $mmoll^{-1}$ (prepared from a calcium atomic absorption standard solution as obtained from Sigma chemical company) were diluted 1:25 with 1% Lanthanum chloride in autoanalyser cups and assayed on a IL I5I Atomic Absorption Spectrophotometer at wavelength 422.7 nm.

Ascorbic Acid

Research has shown that the most reliable estimate of the tissue status and metabolic turnover of ascorbic acid (AA) in normal individuals is obtained when the plasma ascorbic acid levels are assessed in relation to leucocyte ascorbic acid concentrations (Molloy and Wilson, 1980). The plasma AA concentration influences the absorption and discharge of AA from the leucocytes and other tissues into which it can pass. This reversible flow means that to obtain an accurate assay of both plasma and leucocyte levels in blood, steps must be taken to separate the two within 15 minutes of taking a blood

sample. If a longer time period elapses, plasma levels are likely to show a falsely high reading due to a flow of AA from the leucocytes.

The implications of this are clear when taking blood on a high upland moorland, remote from any laboratory. To counteract the problem, a makeshift laboratory equipped with the necessary materials was constructed in a horsebox, and connected to an electricity supply using several hundred metres of electric cable.

Trial runs of the methodology involved were carried out using blood from sheep going for slaughter and from some housed at the Animal Breeding Research Organisation, Edinburgh. The preparation, organisation and transportation of laboratory equipment and materials for this operation was considerable. Because of this, the decision was taken to blood sample lambs at eight weeks of age only - the time when most show the worst symptoms of the locomotor disorder in the affected area. Ideally, lambs would have been sampled in each area at the previously outlined ages, but such a task proved to be practically impossible due to limited loan facilities for equipment.

The following method was employed to separate the leucocytes from whole blood, within the required time specifications (Carlson and Kaneko, 1973). 8 ml of whole blood were taken from each lamb to give two 3 ml samples for duplicate analysis. To each 3 ml of blood pipetted into a sterile 100 ml Pyrex beaker, 9 ml of distilled water was added and a period of 30 seconds allowed to elapse (timed using a stop watch). With all red cells lysed though osmosis, 3 ml of 3.5% NaCl was added to restore isotonicity, and to prevent the leucocytes from lysing. 5 ml of this solution was then removed by calibrated pipette and placed in a capped tube for transportation on ice to a local hospital's Haematology

Department for a duplicate white blood cell count. The remaining 10 ml were centrifuged at 2,200 g for 15 minutes to sediment the leucocytes and the supernatant then poured off. At this point 1.3 ml of 5% Trichloro acetic acid (TCA) was added and the leucocyte pellet 'Rotamixed' for two minutes and then homogenised using a glass rod for 15 seconds to liberate the AA.

The remaining 2 ml of whole blood was used to determine plasma AA levels. The blood was centrifuged at 2200 G for 15 minutes and 0.5 ml plasma taken in duplicate by calibrated pipette into two clean tubes. 2 ml of 5% TCA were added to each, mixed well and capped (Devgun et al, 1981; Denson and Bower, 1961). Throughout this fieldwork, samples were kept in crushed ice and then transferred to a freezer to await the final analysis.

In the laboratory, AA levels in both leucocytes and plasma were assayed colorimetrically (Devgun et al, 1981). The precipitate in each tube was spun off and 1 ml of the supernatant removed for the test. 0.3 ml of colour reagent, comprising 100 ml 2.2% 2.4 dinitrophenyl-hydrazine in 10N H_2SO_4, 5 ml 5% thiourea and 5 ml 0.6% $CuSO_4$, was added to each tube, mixed well on a 'Rotamixer', then capped and incubated at 37°C for 4 hours. The tubes were cooled on ice after incubation and 1.5 ml 65% H_2SO_4 added, mixed and incubated for a further 5 minutes at 37°C.

The final analysis was made using a Pye Unicam SP6 colorimetric spectrophotometer at a wavelength of 520 nm. Standards were prepared in the range of 1 to 5 $\mu g ml^{-1}$ AA in a 5% TCA solution, with 5% TCA used as a blank, and underwent the same analytical process as the samples. Leucocyte AA is expressed in μg per 10^8 white blood cells, while plasma AA is expressed in $\mu g ml^{-1}$ under 'Results'.

Ewe's milk

In view of the elevated blood lead levels in adult sheep shown in previous work in old lead mining areas (Butler et al, 1957; Stewart and Allcroft, 1956) the question of possible lead contamination of ewe's milk and transfer to the lamb was investigated.

Between 20 and 50 ml of milk were taken from the mothers of lambs aged 5-7 days on both farms. Before sampling, each udder was washed using distilled water in order to avoid possible contamination from dust or dirt. No detergents or cleaning agents were employed for fear of the lamb rejecting its mother through an altered body scent. Using latex gloves, the milk was drawn into a sterile container, capped and placed on ice before freezing to await analysis. The analytical procedure for lead in ewe's milk was derived from a method published by the Department of the Environment (1982).

A solution of 1 g ammonium tetra methylene dithiocarbamate in 100 ml of distilled water was prepared and filtered through acid washed filter paper to remove any particulate matter. 1 ml of this solution and 1 g of ewe's milk were taken and brought to pH5 with 3% v/v acetic acid; the pH was checked with narrow range indicator paper (Whatman). 2 ml of 4-methylpentan-2-one (methyl isolbutyl ketone) were added to this solution, and mixed well on a Whirlimixer for 60 seconds. The resultant emulsion was centrifuged at 3000 G for 10 minutes to remove all residual particulate material, with lead extracted into the upper organic layer. This upper layer was injected directly into a Varian AA-1475 Series Atomic Absorption Spectrophotometer fitted with a GTA-95 carbon furnace attachment and using a wavelength of 283.3 nm. Lead in each milk sample was determined in duplicate.

Results were calculated from a standard curve prepared from spiked samples of ewe's milk. For this, one milk

sample was picked at random and 3 aliquots spiked with standards prepared from a standard lead acetate solution to contain 0.032, 0.064 and 0.128 $\mu gPbml^{-1}$. The use of this technique provided standards which were closely matched to the sample with respect to chemical and physical properties. A fourth aliquot of milk had distilled water added in place of a standard. Like the samples, the absorbence of each standard was determined as outlined above and a calibration graph plotted. The slope of the calibration curve was extrapolated to zero absorbence to give the concentration of lead in the sample by the method of additions.

Tissues

Immediate notification was requested from the farmers in both areas on finding a dead lamb, so that samples of bone, liver and kidney could be removed for analysis. Wearing disposable examination gloves and using a scalpel, a total of 13 lambs aged between 1 and 8 weeks were dissected and the liver and kidneys removed and sealed separately in plastic bags. The tibia was taken from the right back leg and also sealed in a polyethylene bag.

Retrieving dead lambs was not easy on the Wanlockhead farm on its expansive upland moorland site. Despite the known high death rate, many carcasses were not found or were found too late in what was an exceptionally warm and sunny spring. As the next chapter shows half the lambs which were blood sampled were missing - presumed dead - by the 12th week of sampling, and only one carcass was retrieved.

The techniques employed for the analysis of heavy metals i.e. lead, zinc and copper in livers and kidneys are as outlined later for herbage samples. Lead, zinc and copper levels in dried bone were determined by taking a section

of bone around 2 mm thick from the centre of the tibia and digesting in a glass vial over a digestion block with 1 ml of nitric acid. Taken almost to dryness, each sample was taken up in 5% hydrochloric acid, made to volume (10 ml) with distilled water and assayed against standards as outlined for herbage samples.

Bone calcium and phosphorous levels were also determined. For calcium, a section of bone was digested as outlined above, whereupon the method followed that given for plasma calcium levels, except that the dilution with Lanthanum chloride was 1:200 rather than 1:25. Standards were again diluted 1:25.

For phosphorus, a colour reaction similar to the principles outlined for plasma phosphorus was employed. 2 ml of the digest solution was added to 5 ml of molybdate solution (25 g ammonium molybdate and 300 ml 10N H_2SO_4 made up to 1 l with distilled water and diluted 1:5 for the working reagent) along with 2 ml amino napthol sulphonic acid reagent (292.5 g sodium metabisulphite and 5 g 1-amino-2napthol-4sulphonic acid dissolved in 1600 ml distilled water. 10 g of sodium sulphite was added and allowed to stand overnight in a darkened area, before making to a 2 l volume with distilled water (diluted 1:5 for working reagent). Approximately 1 ml of distilled water was added to the rest of the sample solution in a tube to make to 10 ml volume. The blue colour was allowed to develop over a 15 minute period and samples and standards assayed as described under plasma phosphorus.

Soil and Herbage

Soil and herbage samples were taken from approximately 200 acres of upland moorland in Wanlockhead and Moniaive grazed by the flocks of sheep. A stratified random sampling procedure was implemented, splitting the hill land into five areas according to elevation. Within each

area samples were collected following a continuous random 'W' pattern to traverse the area (Wilson and Stevens, 1981). The results therefore represent 5 pooled samples and 30 individual samples for both soils and herbage.

Soils were sampled using a stainless steel trowel to a depth of approximately 15 cm and sealed in large polyethylene bags for return to the laboratory. Herbage samples were usually cut with stainless steel shears 5 cm above ground level in order to avoid contamination from soil splash and sealed in polyethylene bags. Assistance was required so that soils and herbage could be sampled together, but without risk of contamination. One person therefore sampled herbage and the other soils. Latex gloves were worn throughout.

Soil samples were analysed for the total concentrations of the heavy metals, lead, zinc and copper and for the extractable macronutrients, phosphorus, calcium and sulphur.

The sample preparation procedures for lead and copper were as outlined for 'Lead in Garden soils'. Zinc analysis also followed this method initially; however after filtration, 5 ml of the filtrate was diluted to 20 ml and concentrations of zinc were determined by atomic absorption as before. Lead standards were prepared as previously outlined while standards for zinc and copper were prepared from BDH stock solutions of $ZnSO_4$ and $CuSO_45H_2O$ to contain 1, 2, 4, 8, 16 and 32, and 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 μgml^{-1}, respectively. Heavy metals were determined on an IL I5I Atomic Absorption Spectrophotometer with an air-acetylene flame at a wavelength of 217.0, 324.7, and 213.9 nm for lead, copper and zinc, respectively.

For phosphorus and calcium determinations, 5 g of air dried soil was put into a 300 ml polyethylene bottle and 150 ml of distilled water and 50 ml of 1.72 M acetic acid

added. The bottle was then capped and placed on an orbital shaker for 2 hours at 1800 r.p.m. After extraction, a 7.5 ml aliquot of each sample was centrifuged at 1000 G for 1 minute. The resultant clear extract was used for the eventual determination of phosphorus and calcium. For phosphorus, 4000 μl of ammonium molybdate working solution was added to 500 μl of sample and 10 minutes allowed to elapse, before measuring the full colour development at 880 nm on an SP6 Colorimeter. For calcium, 5000 μl of a strontium releasing agent was dispensed into 175 μl of sample. Each solution was then aspirated into an ILI 251 atomic absorption spectrophotometer. Standards were prepared from Analar solutions of $NH_4H_2PO_4$ and $CaCO_3$ and underwent a similar digestion procedure as above : they contained 0.42, 1.25, and 2.5 $mg50ml^{-1}$ PO_4 and 0.70, 2.10, and 4.20 $mg50ml^{-1}$ Ca, respectively.

For the estimation of sulphate sulphur in soils, air dried soil was ground to pass a 2 mm sieve, and 2 x 10 ml scoops placed in a 100 ml polyethylene bottle to which 50 ml of extracting solution [$Ca(H_2PO_4)_2.2H_2O$] was added and shaken on an orbital shaker for 30 minutes. Filtered through a 9 cm G.F.A. filter, 32 ml of filtrate was put into a digestion tube and evaporated to near dryness on a Tecator BD40 block digester set at 100°C. 4 ml of Analar nitric/perchloric acid digestion mix (4:1 v/v) was then added and the block digester temperature raised firstly to 150°C for digestion over 30 mins., and secondly to 180°C to drive off any remaining nitric acid. On cooling, 16 ml of *mixed acid reagent was added (* this comprised 500 ml distilled water, 150 ml glacial acetic acid, 60 ml 85% orthophosphoric acid, 60 ml 35% hydrochloric acid and 2.6 ml of stock 7.0 $mgml^{-1}$ SO_4:S solution, made to 1 litre volume with distilled water. The stock solution was made by dissolving 0.2783 g Analar potassium sulphate in 100 ml distilled water.

4 ml of the digest was transferred to a 6 ml analyser cup and placed in position on the turn-table of a T40 sample processing station which allows for stirring and timing facilities as well as regulating reagent addition. 2 ml of $BaCl_2$: polyacrylamide solution (the precipitating reagent) was dispensed into each cup and allowed to stand for 15 minutes for the precipitate fully to develop. Each cup was then agitated using a stirrer and the turbidity measured at 653 nm on a Pye Unicam SP6-500. Standards were prepared from the stock solution to contain 4, 12, 20 and 24 μgml^{-1} SO_4 and subjected to the same procedure as the samples.

Herbage samples were mixed well, freeze dried for 24 hours and subsequently milled to a fine particle size using a Hammer Mill, for the analysis of the total heavy metals lead, zinc and copper. 0.5 g of the milled material was placed in a glass digestion vial and 5 ml of nitric/perchloric acid mix (4:1) added. Samples were digested on a digestion block and taken to dryness before being taken up in 5 ml 5% hydrochloric acid. Standards were again prepared using BDH stock standard solutions in 5% hydrochloric acid containing 0.2, 0.6, 1.0, 1.4 and 1.8 μgml^{-1} or 1.0, 2.0, 3.0, 4.0 and 5.0 μgml^{-1} for zinc and copper, and 0.2 to 1.0 μgml^{-1} or 2.5 to 10 μgml^{-1} lead, as appropriate. Samples and standards were assayed using an IL I5I Atomic Absorption Spectrophotometer, as described for blood analysis.

For total calcium and phosphorus analyses, 250 mg of dried milled plant material (oven dried at 80°C) was weighed into a 50 ml graduated digestion tube and 5 ml of digestion acid added (nitric/perchloric acid, 4:1 v/v). The contents were mixed thoroughly on a Whirlimixer and allowed to stand overnight in order to avoid severe frothing or ignition on heating. Tubes were then placed in a digestion block preheated to 150°C, and boiled for 30 minutes. After the dissolution period, the temperature

was raised to 180°C to boil off excess nitric acid, and continuing to heat at this temperature the solution volume was reduced to approximately 1 ml. On cooling, 45 ml of hot distilled water was added, the solution cooled once more and finally made to 50 ml volume with distilled water. The final determination of these elements was as outlined in the soil method for calcium and phosphorus.

For total sulphur analysis, 500 mg of the herbage sample was weighed into a 50 ml digestion tube and 200μl of catalyst (potassium dichromate/ammonium metavanadate) and 8 ml of the acid digestion mix added. Each tube was shaken and allowed to stand overnight before placing in an aluminium digestion block preheated to 120°C. The initial reaction having subsided, the block temperature was firstly increased to 150°C for 1 hours digestion and secondly to 180°C for 1 hour to boil off the nitric acid. The digests were then removed, cooled, and made to 50 ml volume with distilled water. For analysis, 2 ml of the diluted digest was analysed according to the soil sulphur method, but with only 1.2 ml of stock 7.0 $mgml^{-1}$ SO_4: S solution added to the mixed acid reagent; standards containing 1.4, 4.2, 7.0 and 8.4 gl^{-1} sulphur were used.

3.2.3 Experiment 2

An experiment was set up to study the effects on the locomotor disorder in lambs of dietary supplements of ascorbic acid, and calcium and phosphorus in May 1985. Twenty-four Blackface lambs aged between 4 and 7 days old were chosen at random from the farm used in Experiment 1 and split into three equal groups of eight lambs according to sex and age. Group numbers were chosen according to previous research (e.g. Quarterman et al, 1977) with the final allocation to each group formed by random number tables (Armitage and Berry, 1971, 1987). The lambs and their dams were transferred to a farm in Leadhills where fenced off pasture and catching pens were

available for easy handling. The Leadhills farm was also known to sustain heavy lamb losses each year (Moffat, 1982).

Group 1 were given 1.5 g calcium and 1 g phosphorus daily for 14 days (Figure 11a). The supplement was prepared from calcium gluconate and anhydrous disodium hydrogen ortho phosphate salts. Each lamb received 16.8 g and 4.6 g of the salts, respectively, dissolved in 100 ml distilled water by means of a lamb feeder and stomach tube. Group 2 received 1 g ascorbic acid daily for 14 days in the form of an effervescent tablet dissolved in 10 ml distilled water. The tablets were obtained from a chemists shop and administered using an oral syringe (Figure 11b). Group 3 received no dietary supplement and acted as control.

Every lamb was blood sampled immediately before the dosing programme began and then at 7 days and 14 days whereupon all dosing was ceased. Restrictions set by the farmer coupled with unavoidably limited veterinary help meant that the programme could not be extended beyond 14 days, half the intended time period.

Field and laboratory techniques and estimations were implemented as previously outlined for the determination of lead in whole blood, and plasma ascorbic acid. Plasma iron levels were assayed using the same blood sample and the laboratory technique previously described for plasma zinc and copper. Iron standards containing 1.0, 2.0, 3.0, 4.0 and 5.0 $\mu g ml^{-1}$ were employed.

3.2.4 Analysis, Sampling And Control Of Bias

Blood analysis for heavy metals and macronutrients was carried out by the Moredun Research Institute, Edinburgh under the charge of N.F. Suttle.

This laboratory also undertook the analysis of heavy metals in herbage samples and all tissue analyses. The estimation of ascorbic acid in blood and lead in ewe milk was determined by the author in the East of Scotland College of Agriculture laboratories. Work on macronutrients in herbage as well as heavy metals and macronutrients in soils was also carried out in the latter laboratory run by P. Crooks and D. Purves.

All blood samples taken from sheep and lambs over the two year period were subject to 'blind duplicate' sampling. This varied from 1 in 5 to 1 in 10 samples a procedure which the analyst was left unaware of through coding procedures. Ewe milk samples were all analysed in duplicate, again using an unknown coding system. One in five tissues of liver, kidney and bone, and soil and herbage samples were re-analysed as a check on laboratory reproducibility. Reagent blanks, and where possible inhouse/certified reference materials were also used.

3.2.5 Statistical Methods

As with the human data, checks were carried out on each data set to confirm the presence of normal distribution before applying parametric tests. Logarithm transformation was used where data sets were skewed, before applying analysis of variance or a weighted paired T-test as appropriate (i.e. two-way presentations of results where changes within individuals minimises individual influence) to test for significant differences between populations: this was done using Minitab (Ryan et al, 1978). Pearson's correlation analysis was used to determine the strength of relationships between variables.

3.3 RESULTS

EXPERIMENT 1:

3.3.1 Mineral Concentrations In Ewe Blood

The mean ewe blood levels of lead, copper, zinc, phosphorus and calcium from 21 ewes in Wanlockhead and 13 ewes in Moniaive are displayed in Table 20 (factors for converting conventional to S.I Units are shown separately in Table 21).

There was a striking difference in blood lead ($p<0.001$) between farms; ewes in Wanlockhead had a geometric mean of 0.42 μgml^{-1}, compared to those in Moniaive of 0.17 μgml^{-1}, an increase of 147 percent. By contrast ewes in the high lead area had a mean copper level of 0.61 μgml^{-1} which was 30% lower than the mean found in the control farm of 0.88 μgml^{-1} ($p<0.001$). Mean zinc, calcium and phosphorus levels in blood showed no significant difference between the farms.

3.3.2 Lead Concentrations In Ewe Milk

The results of lead analysis in ewe milk are presented in Table 22. A significant difference at ($p<0.001$) was observed between the two farms, the geometric mean of 0.14 μgml^{-1} at Wanlockhead being some five fold greater when compared to the present control value of 0.03 μgml^{-1}. Lead concentrations in milk were far lower than those in blood on both farms.

TABLE 20

Mean concentrations of ewe blood (sd or 95% range) on the two farms - lead, copper, zinc, phosphorus and calcium.

	[1]LEAD (μgml^{-1})		COPPER (μgml^{-1})		ZINC (μgml^{-1})		PHOSPHORUS ($mmoll^{-1}$)		CALCIUM ($mmoll^{-1}$)	
	n	Mean (95% range)	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)
WANLOCKHEAD	21	0.42 (0.38-0.46)	21	0.61 (0.08)	21	0.58 (0.09)	21	1.58 (0.36)	21	2.14 (0.11)
MONIAIVE	13	0.17 (0.16-0.19)	13	0.88 (0.22)	12	0.51 (0.10)	13	1.77 (0.26)	13	2.14 (0.20)
		***		***						

Note 1: Blood lead geometric mean and 95% confidence limits obtained after log transformation.

2: Analysis of variance used to test for significance, ***$p<0.001$

TABLE 21

Conversion factors for conventional to SI units in blood and tissue analyses.

Component	Conventional Unit	x factor	SI Unit
Lead	$\mu g100ml^{-1}$	0.0483	$\mu moll^{-1}$
Copper	$\mu g100ml^{-1}$	0.1574	$\mu moll^{-1}$
Zinc	$\mu g100ml^{-1}$	0.1530	$\mu moll^{-1}$
Iron	$\mu g100ml^{-1}$	0.1791	$\mu moll^{-1}$
Phosphorus	$mg100ml^{-1}$	0.3229	$mmoll^{-1}$
Calcium	$mg100ml^{-1}$	0.2495	$mmoll^{-1}$

Note: Present results for heavy metals are listed as μgml^{-1} and macronutrients as $mmoll^{-1}$

TABLE 22

Geometric mean ewe milk lead levels on the two farms.

MILK LEAD ($\mu g ml^{-1}$)

	n	Geometric Mean (95% range)
WANLOCKHEAD	15	0.14 (0.12-0.17)
MONIAIVE	13	0.03 (0.02-0.04) ***

Note 1: Log transformation used.
2: Analysis of variance used to test for significance, ***$p<0.001$

3.3.3 Mineral Concentrations In Lamb Blood

From an initial population of 24 lambs first sampled at 1 week of age in Wanlockhead, nine lambs showed stiffness of gait and one had died by 4 weeks of age. By 8 weeks of age eight lambs were stiff, one had posterior paralysis and five had died, and by 12 weeks of age seven lambs were stiff and 12 had died: some affected lambs had diarrhoea (Figure 12). On the control farm 18 lambs were sampled throughout and no deaths occurred. The mean blood levels in the two areas for lead, copper, zinc, phosphorus and calcium for lambs of different ages are displayed in Figures 13 to 17, respectively, and Appendix 1. Ascorbic acid concentrations are shown in Appendix 1. Tests of variance on the means of the two groups were carried out as above, with the exception of lead. This was determined using a two-sample T-test which was weighted to allow for the different variances observed.

Lead

There were striking differences ($p<0.001$) between populations at all ages. At 1 week of age, lambs in Wanlockhead had a mean value of 1.09 μgml^{-1} compared with 0.23 μgml^{-1} in Moniaive. By 4 weeks of age the mean level in Wanlockhead had risen to 1.45 μgml^{-1} but remained constant in Moniaive. Thereafter, values declined on both farms but by twelve weeks were still far higher in Wanlockhead than on the control farm (0.67 μgml^{-1} and 0.15 μgml^{-1}, respectively). Figure 13 and Table 23 clarify the significance in lamb blood lead between successive age groups on each farm, highlighting the rise in blood lead in Wanlockhead lambs between the ages of 1 and 4 weeks ($p<0.001$), and the subsequent falls between 4 and 8 weeks ($p<0.05$ and $p<0.001$) and 8 and 12 weeks ($p<0.001$ and $p<0.05$) on the Wanlockhead and Moniaive farms, respectively.

a.

b.

b.

c.

d.

Figure 12a. Posterior Paralysis Caused by Collapse of a Lumbar Vertebra.

Figure 12b. "Stiff" Lambs Caused by Brittleness and Breaks in Bones.

Figure 12c. Lameness Produced by Front Limb Fractures.

Figure 12d. Lamb showing Diarrhoea.

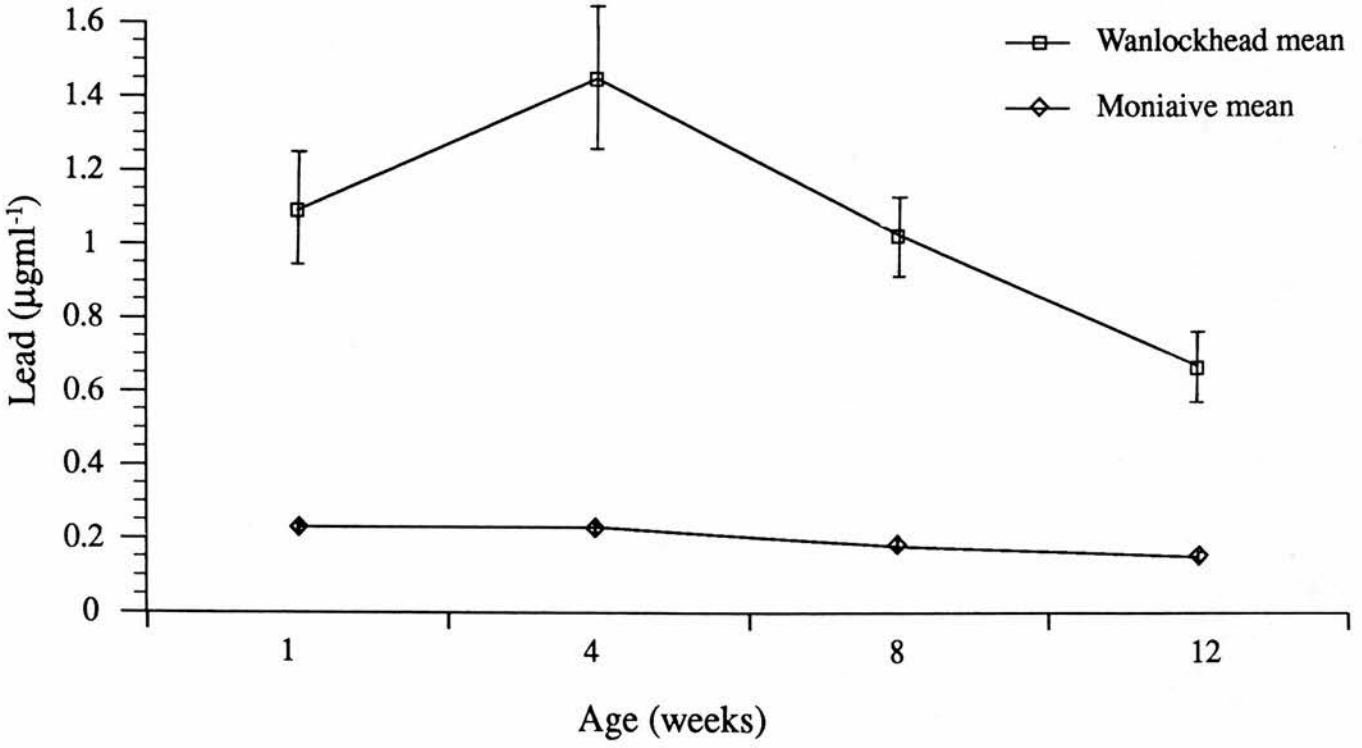


Fig.13: Mean (s.e.) lamb blood lead concentrations on the two farms

TABLE 23

Mean comparisons in lamb bloods between successive age groups on each farm. (Paired t-test used to test for significance, where * $p<0.05$, ** $p<0.01$, and *** $p<0.001$).

	AGE OF LAMB IN WEEKS					
	1 and 4		4 and 8		8 and 12	
	n	Mean (se)	n	Mean (se)	n	Mean (se)
LEAD ($\mu g\ ml^{-1}$)						
Wanlockhead	23	+0.41(0.107)	18	-0.37(0.141)	12	-0.40(0.066)
		***		*		***
Moniaive	18	0.00(0.012)	18	-0.05(0.007)	18	-0.03(0.012)
				***		*
COPPER ($\mu g\ ml^{-1}$)						
Wanlockhead	23	+0.01(0.040)	18	+0.22(0.062)	12	-0.15(0.087)
				**		
Moniaive	18	+0.20(0.076)	18	+0.08(0.070)	18	+0.08(0.055)
		*				
ZINC ($\mu g\ ml^{-1}$)						
Wanlockhead	23	+0.05(0.040)	18	+0.08(0.028)	12	-0.07(0.027)
				**		*
Moniaive	18	-0.19(0.057	18	+0.07(0.020)	18	+0.14(0.019)
		**		**		***

Continued overleaf .....

TABLE 23 (contd.)

	AGE OF LAMB IN WEEKS					
	1 and 4		4 and 8		8 and 12	
	n	Mean (se)	n	Mean (se)	n	Mean (se)
PHOSPHORUS (mmol l^{-1})						
Wanlockhead	23	+1.14(0.265)	18	-1.05(0.264)	12	-0.44(0.203)
		***		***		*
Moniaive	18	-0.60(0.074)	18	+0.87(0.082)	18	+0.25(0.268)
		***		***		
CALCIUM (mmol l^{-1})						
Wanlockhead	23	-0.20(0.064)	18	+0.32(0.055)	12	-0.34(0.106)
		**		***		**
Moniaive	18	-0.28(0.041)	18	-0.02(0.047)	18	-0.44(0.091)
		***				***

Mean lamb blood lead levels are tabulated in Table 24 according to presence or absence of a locomotor disorder at 4, 8 and 12 weeks of age. Affected lambs aged 4 weeks showed a significantly greater ($p<0.05$) blood lead compared to normal lambs. Table 25 further examines blood lead at the early ages of 1 and 4 weeks and the later occurrence of normal locomotion, stiffness or death at 8 and 12 weeks of age. Although there was a tendency for the most clinically affected lambs to have the highest blood lead values, no significant differences were evident. Finally, no significant difference was observed in blood lead at any age according to sex (Table 26). From an original group of thirteen female and eleven male lambs, five males and four females were stiff by 4 weeks of age, and one male had died; by 8 weeks, seven males and two females were stiff, while three males and two females had died; and finally by 12 weeks of age, four males and three females were showing signs of stiffness, while four males and eight females were dead.

Copper

The copper level of 0.64 $\mu g ml^{-1}$ in 1 week old Wanlockhead lambs was significantly different ($p<0.05$) from that in Moniaive where a level of 0.53 was monitored. No differences were found between populations at 4 and 8 weeks but there was a general rise in plasma copper on both farms. However by 12 weeks of age plasma copper levels were significantly lower in Wanlockhead lambs with a mean of 0.69 $\mu g ml^{-1}$ when compared to the control value of 0.91 $\mu g ml^{-1}$ ($p<0.01$). Figure 14 and Table 23 supplement this observation, showing a significant rise in copper levels in Wanlockhead lambs alone between the ages of 4 and 8 weeks ($p<0.01$).

TABLE 24

Mean lamb blood lead levels ($\mu g ml^{-1}$) at different ages in Wanlockhead, according to clinical condition score (where 1 = normal locomotion and 2 = stiffness).

AGE	CLINICAL CONDITION SCORE	n	Mean	(sd)
4 weeks	1.	15	1.14	(0.47)
	2.	8	2.05*	(1.20)
8 weeks	1.	9	0.94	(0.38)
	2.	9	1.11	(0.46)
12 weeks	1.	5	0.54	(0.14)
	2.	7	0.76	(0.37)

Note 1: Analysis of variance used to test for significance, where *$p<0.05$.

TABLE 25

Mean blood lead levels ($\mu g ml^{-1}$) in Wanlockhead lambs at 1 and 4 weeks of age, according to the later development of disorders at 8 and 12 weeks of age (where 1 = normal locomotion, 2 = stiffness, and 3 = death).

	Blood lead at 1 week			Blood lead at 4 weeks		
	n	Mean	(sd)	n	Mean	(sd)
CLINICAL CONDITION						
at 8 weeks						
1.	9	1.17	(0.99)	9	1.41	(1.08)
2.	9	1.00	(0.42)	9	1.37	(0.47)
3.	6	1.12	(0.80)	5	1.67	(1.23)
at 12 weeks						
1.	5	0.80	(0.08)	5	1.09	(0.28)
2.	7	1.07	(0.44)	7	1.36	(0.54)
3.	12	1.23	(0.99)	11	1.67	(1.19)

Note 1: Analysis of variance and Mann Whitney tests found no statistical significance.

TABLE 26

Mean lamb blood lead levels ($\mu g ml^{-1}$) in Wanlockhead, according to sex at different ages.

AGE	sex	n	Mean	(sd)
1 week	male	11	1.01	(0.47)
	female	13	1.16	(0.92)
4 weeks	male	10	1.22	(0.52)
	female	13	1.63	(1.08)
8 weeks	male	8	1.13	(0.49)
	female	10	0.94	(0.36)
12 weeks	male	7	0.77	(0.36)
	female	5	0.52	(0.15)

Note 1: Analysis of variance tests found no statistical significance.

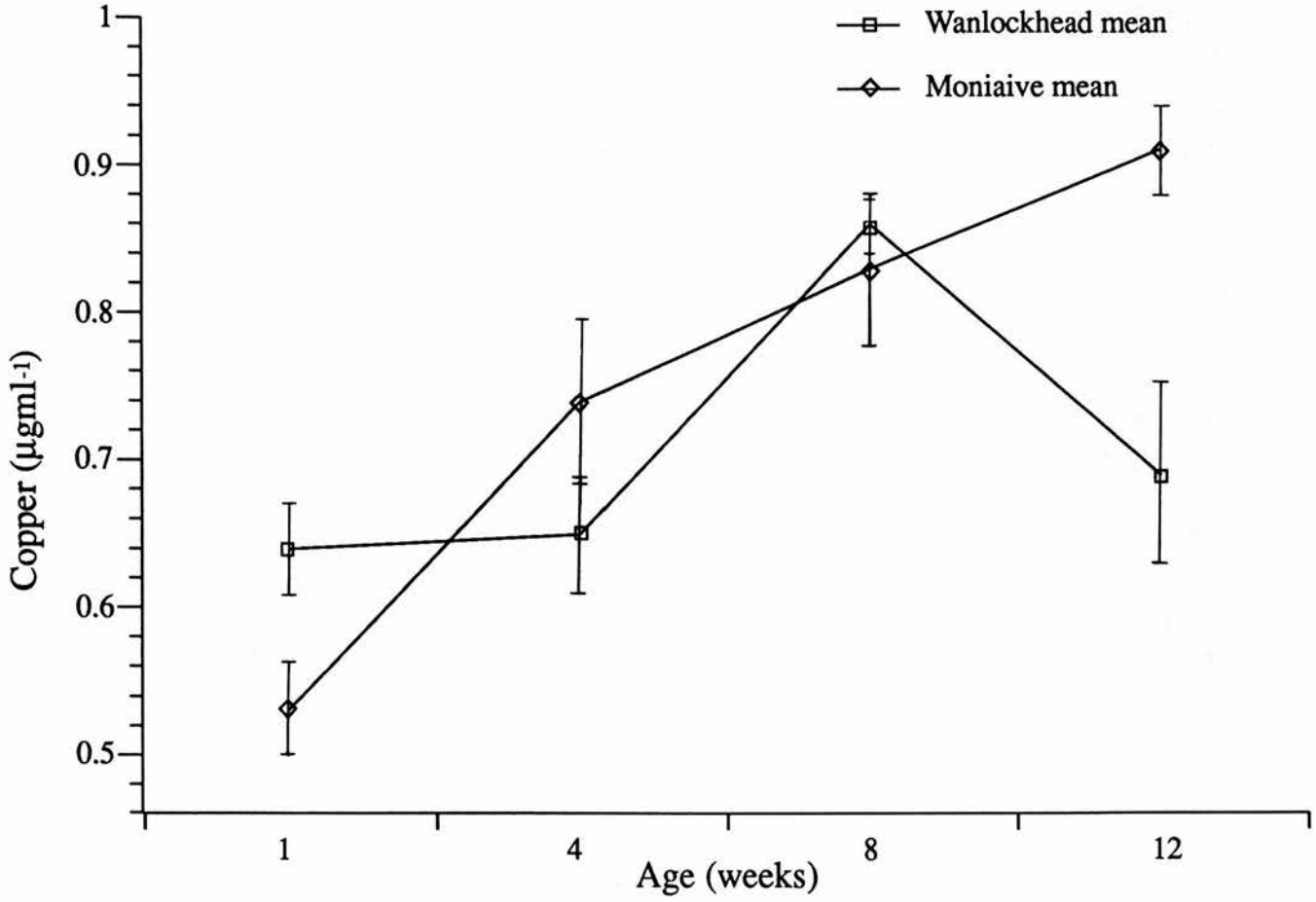


Fig.14: Mean (s.e.) lamb plasma copper concentrations on the two farms

Zinc

Zinc plasma levels at one week of age were similar on both farms but by 8 and 12 weeks mean levels had increased in Wanlockhead to 0.82 μgml^{-1} and 0.89 μgml^{-1} ($p<0.001$ and $p<0.001$, respectively) compared to decreased values of 0.69 μgml^{-1} and 0.76 μgml^{-1} in Moniaive. By 12 weeks of age Moniaive lambs had slightly higher plasma levels of zinc ($p<0.05$) compared to the former lead mining area. Statistically significant differences were observed between age groups in lambs on both farms (Figure 15 and Table 23).

Phosphorus

Phosphorus levels showed no significant differences between farms in 1 week old lambs but mean plasma phosphorus concentrations in Wanlockhead had increased by 4 weeks of age to 3.66 $mmoll^{-1}$ a value well above the normal limit of 2.70 $mmoll^{-1}$. Mean plasma phosphorus concentrations then fell to 2.70 $mmoll^{-1}$ ($p<0.01$) and 2.21 $mmoll^{-1}$ ($p<0.01$) by 8 and 12 weeks of age, respectively, to become significantly lower than those at Moniaive. An analysis of changes between age groups (Table 23) showed a significant rise between 1 and 4 weeks and then falls in phosphorus between 4 and 8 weeks ($p<0.001$) and 8 and 12 weeks ($p<0.05$) in Wanlockhead lambs. Over the same periods, phosphorus in lambs in Moniaive decreased and then increased, although only the last change was not significant ($p<0.001$). A graphical illustration of these results is shown in Figure 16.

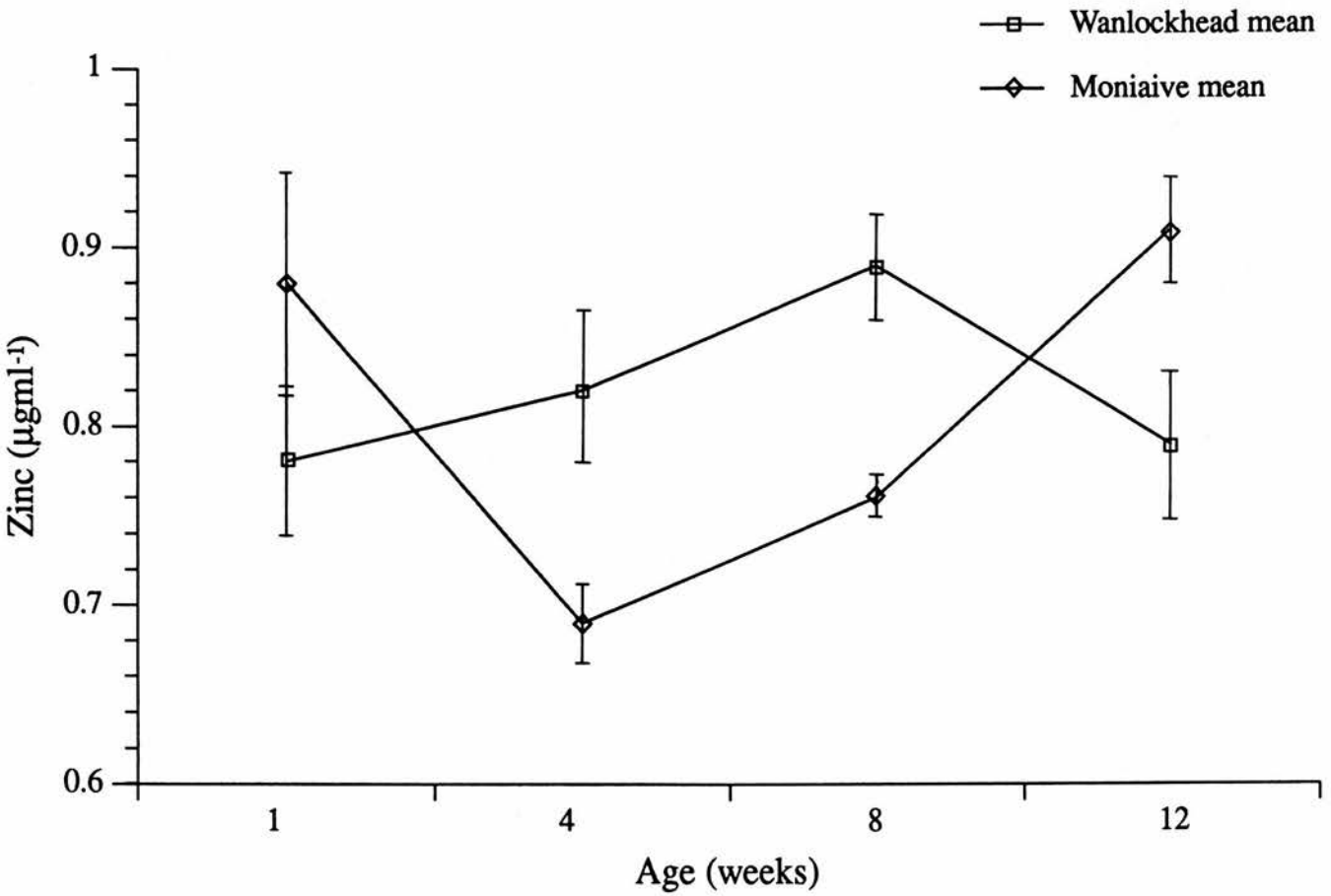


Fig.15: Mean (s.e.) lamb plasma zinc concentrations on the two farms

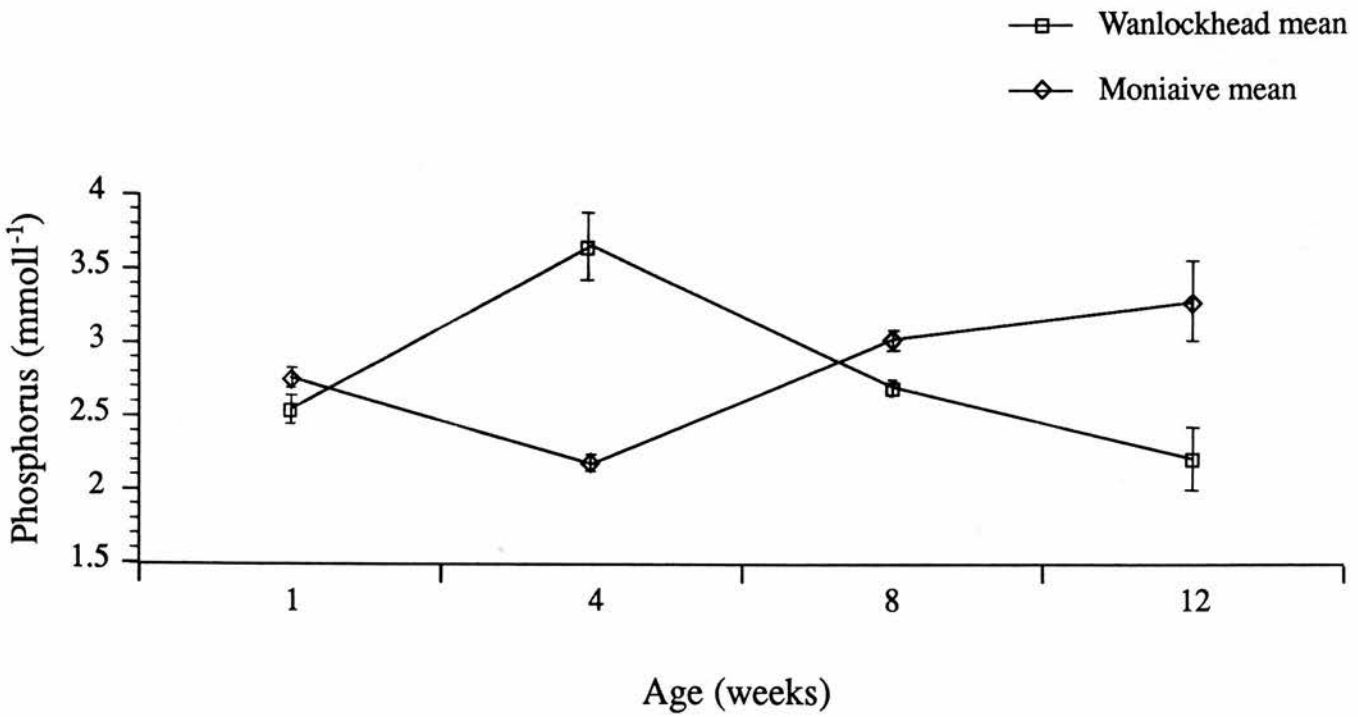


Fig.16: Mean (s.e.) lamb plasma phosphorus concentrations on the two farms

Calcium

Statistically significant differences were evident at 1 and 4 weeks when Wanlockhead lambs exhibited low plasma calcium concentrations compared to the control values ($p<0.001$ and $p<0.01$, respectively). No such differences between farms was apparent thereafter. Figure 17 and Table 23 depict the differences between age groups on each farm, with a fall in plasma calcium throughout the trials in Moniaive but a rise between 4 and 8 weeks in Wanlockhead.

Ascorbic Acid

For reasons described under 'Methodology', plasma ascorbic acid levels were measured only in lambs at 8 weeks of age. At this stage Wanlockhead lambs had a reduced ascorbic acid level of 8.81 μgml^{-1} compared to the Moniaive value of 10.94 μgml^{-1} ($p<0.01$): Appendix 1.

3.3.4 Tissue Mineral Concentrations

Mean tissue levels of lead, copper and zinc in liver and kidney and of lead, copper, zinc and phosphorus in bone are described in Table 27. Calcium levels are unavailable due to laboratory error.

Liver

Large differences existed for both liver lead and liver copper concentrations between the two areas. The geometric mean for lead was 56.0 μgg^{-1} in Wanlockhead and only 8.6 μgg^{-1} in Moniaive ($p<0.001$), while for copper a far lower geometric mean of 19.4 μgg^{-1} was observed on the Wanlockhead farm compared with 162.9 μgg^{-1} on the control farm. No significant difference was attached to zinc concentrations.

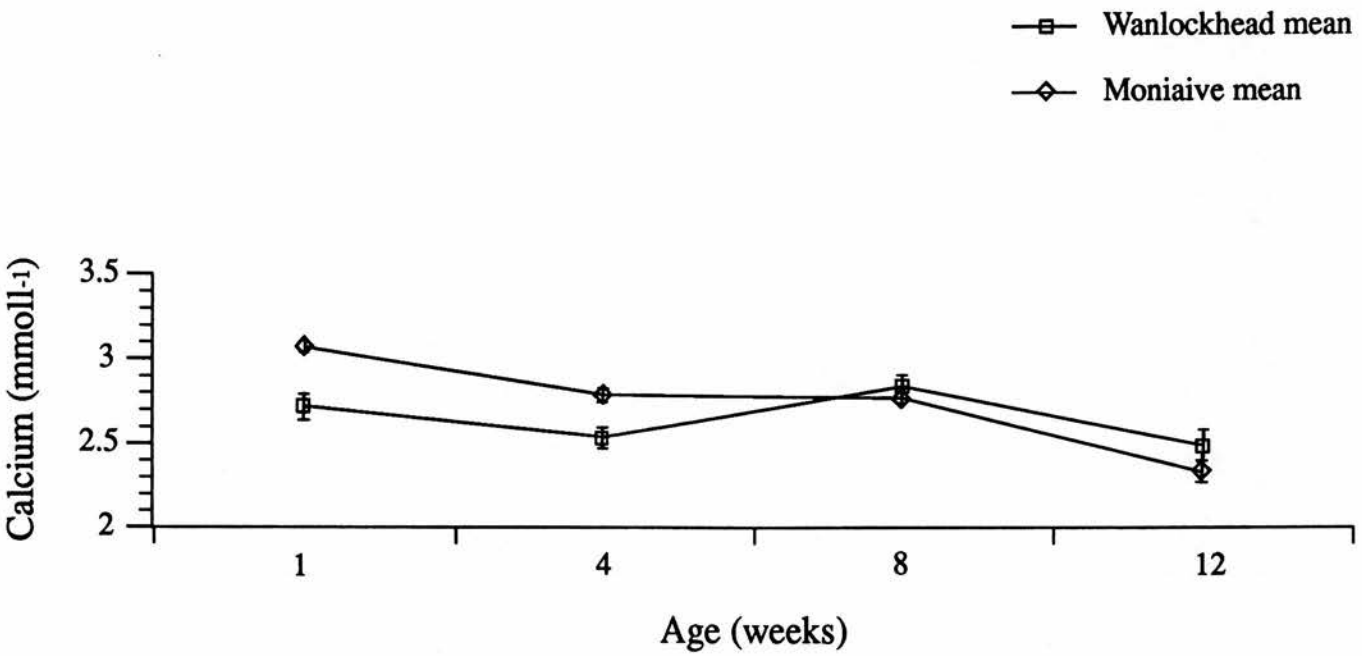


Fig.17: Mean (s.e.) lamb plasma calcium concentrations on the two farms

TABLE 27

Mean tissue levels of lead, copper, zinc, and phosphorus (all DM) in lambs which died on the two farms.

	LEAD (μgg^{-1})		COPPER (μgg^{-1})		ZINC (μgg^{-1})		PHOSPHORUS (%)	
	Mean	(95% range)	Mean	(95% range) (or sd)	Mean	(sd)	Mean	(sd)
1. LIVER								
Wanlockhead	56.0	(35.1-89.3)	19.4	(9.9-37.9)	166.5	(57.7)	-	-
Moniaive	8.6	(6.0-12.4)	162.9	(58.6-452.9)	204.4	(182.3)	-	-
	***		***					
2.KIDNEY								
Wanlockhead	87.6	(36.5-228.0)	13.7	(8.9)	101.6	(33.0)	-	-
Moniaive	1.8	(1.3-2.6)	13.1	(6.8)	85.4	(23.7)	-	-
	***							
3. BONE								
Wanlockhead	190.8	(138.0-260.6)	4.1	(1.4)	99.8	(10.7)	9.6	(1.0)
Moniaive	13.3	(9.8-17.9)	4.7	(2.2)	101.3	(37.2)	9.1	(2.2)
	***							

Note 1: n=8, Wanlockhead; n=5, Moniaive, for each tissue.
2: Geometric means and 95% ranges for liver lead and copper, kidney lead and bone lead obtained after log transformation.
3: Analysis of variance used to test for significance, $^{***}p<0.001$.

Kidney

Kidney analyses indicated a geometric mean of 87.6 μgg^{-1} lead in lambs from the former lead mining area and only 1.8 μgg^{-1} in Moniaive ($p<0.001$). The zinc concentrations tended to be higher at Wanlockhead whereas the copper results were similar on the two farms.

Bone

Lead concentrations in bone from lambs at Wanlockhead were far higher than those in liver and kidney. The geometric mean for bone lead in Wanlockhead was significantly greater ($p<0.001$) than that observed in Moniaive, with geometric means of 190.8 μgg^{-1} and 13.3 μgg^{-1}, respectively. No other differences were observed in bone mineral concentrations.

3.3.5 Soil Mineral Status

Total mean soil levels of lead, copper and zinc, and available levels of phosphorus, calcium and sulphur in the two areas are illustrated in Table 28. Analysis of variance has been used to test for statistical significance, except for zinc and copper levels where a weighted t-test was implemented to accommodate wide variance. Arithmetic ranges are cited throughout.

TABLE 28

Geometric (G) or arithmetic (A) mean total (T) or extractable (E) soil concentrations - lead, copper, zinc, phosphorus, calcium and sulphur on the two farms ($\mu g g^{-1}$ DM).

	Wanlockhead	Moniaive	
LEAD (T)			
Mean (G)	3826	81	***
Range	1422 - 9540	43 - 207	
COPPER (T)			
Mean (G)	91	9	**
Range	14 - 198	8 - 10	
ZINC (T)			
Mean (G)	326	70	
Range	38 - 610	61 - 77	
PHOSPHORUS (E)			
Mean (A)	2.2	2.3	
Range	0.6 - 5.2	1.2 - 3.3	
CALCIUM (E)			
Mean (A)	48	204	***
Range	12 - 76	187 - 219	
SULPHUR (E)			
Mean (G)	12	21	*
Range	9 - 14	12 - 30	

Note 1: Each mean is based on 5 bulk samples per farm.
2: All ranges are Arithmetic.
3: Analysis of variance used to test for significance, where: *$p<0.05$; **$p<0.01$; and ***$p<0.001$.
4: A weighted T-test was used for copper and zinc.

Heavy Metals

An almost fifty fold difference in the geometric mean for soil lead was established between the Wanlockhead value of 3826 μgg^{-1} when compared to 81 μgg^{-1} in Moniaive ($p<0.001$). Geometric mean levels for copper in soils also showed a marked difference with the mean value in Wanlockhead of 91 μgg^{-1}, some ten fold in excess of the control farm at 9 μgg^{-1} ($p<0.01$).

For zinc, a mineral enrichment trend was again apparent with a geometric mean of 326 μgg^{-1} in the former lead mining area compared to 70 μgg^{-1} in Moniaive.

Macro-Nutrients

Mean phosphorus levels in soil were similar on both farms. On the other hand, calcium levels showed a marked contrast (at $p<0.001$) with a mean of 48 μgg^{-1} recorded in Wanlockhead and a four fold higher value in Moniaive (204 μgg^{-1}). Sulphur levels were also relatively low in Wanlockhead with a geometric mean of 12 μgg^{-1} compared to 21 μgg^{-1} in Moniaive ($p<0.05$).

3.3.6 Herbage Mineral Status

Total mean herbage concentrations of lead, copper, zinc, phosphorus, calcium and sulphur on each farm are expressed in Table 29.

Heavy Metals

A highly significant difference ($p<0.001$) was evident for lead in herbage between Wanlockhead and Moniaive farms where geometric means of 42.7 μgg^{-1} and 2.6 μgg^{-1} were found, respectively. Although less dramatic, copper in herbage also showed a significant difference between

farms ($p<0.05$) with a mean of 13.6 $\mu g g^{-1}$ in Wanlockhead, and a lesser value of 11.6 $\mu g g^{-1}$ in Moniaive. There was no significant difference in herbage zinc between farms.

Macro-Nutrients

Only phosphorus showed a significant difference between farms: a value of 2200 $\mu g g^{-1}$ was found in Wanlockhead, and 1500 $\mu g g^{-1}$ in Moniaive ($p<0.01$). Calcium and sulphur contents in herbages from Wanlockhead resembled those from the control farm.

TABLE 29

Total mean herbage concentrations of lead, copper, zinc, phosphorus, calcium and sulphur on the two farms ($\mu g g^{-1}$ DM).

	Wanlockhead	Moniaive	
LEAD			
Mean (G)	42.7	2.6	***
Range	14.5 - 97.9	1.8 - 3.6	
COPPER			
Mean	13.6	11.6	*
Range	13.0 - 14.9	10.2 - 13.1	
ZINC			
Mean	66.1	41.3	
Range	28.9 - 126.5	28.0 - 59.5	
PHOSPHORUS			
Mean	2,200	1,500	**
Range	2,000 - 2,400	1,200 - 2,100	
CALCIUM			
Mean	3,500	2,400	
Range	2,600 - 4,900	2,000 - 3,100	
SULPHUR			
Mean	2,000	2,100	
Range	1,620 - 2,520	480 - 2,760	

Note 1: Each mean is based on 5 bulk samples per farm.
2: G = Geometric mean for Pb.
3: All ranges are Arithmetic.
4: Analysis of variance used to test for significance, where: $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$.

EXPERIMENT 2:

3.3.7 Responses Of Lambs To Vitamin C And Mineral Supplements

Twenty four lambs from Wanlockhead aged between 4 and 7 days old were split into three equal groups of 8 lambs according to sex and age. Group 1 lambs received a daily supplement of 1.5 g calcium and 1 g phosphorus over a 14 day period; Group 2 lambs were given a daily supplement of 1 g ascorbic acid over the same time period; while the third group acted as control and were given no supplement.

Group 1 lambs all survived and showed a marked improvement in locomotor ability compared to groups 2 and 3. After one week, two lambs in Group 2 and three lambs in Group 3 were exhibiting signs of stiffness of gait and slow locomotion. By the end of two weeks treatment, three lambs in Group 2 showed signs of the locomotor disorder and two had died. Four lambs in Group 3 also showed signs of the disorder and one had died. Moreover, the majority of lambs in both Groups 2 and 3 were unable to be herded one mile to their home farm as a result of locomotor disability.

The results for blood lead, ascorbic acid and iron in relation to treatment programmes are set out in Figure 18 and Appendix 2. Neither treatment significantly affected blood lead concentration in lambs which showed a tendency to peak in the first few weeks of life as in the previous study. Nevertheless blood lead increased less markedly in those lambs receiving a calcium/phosphorus supplement compared to the other two groups.

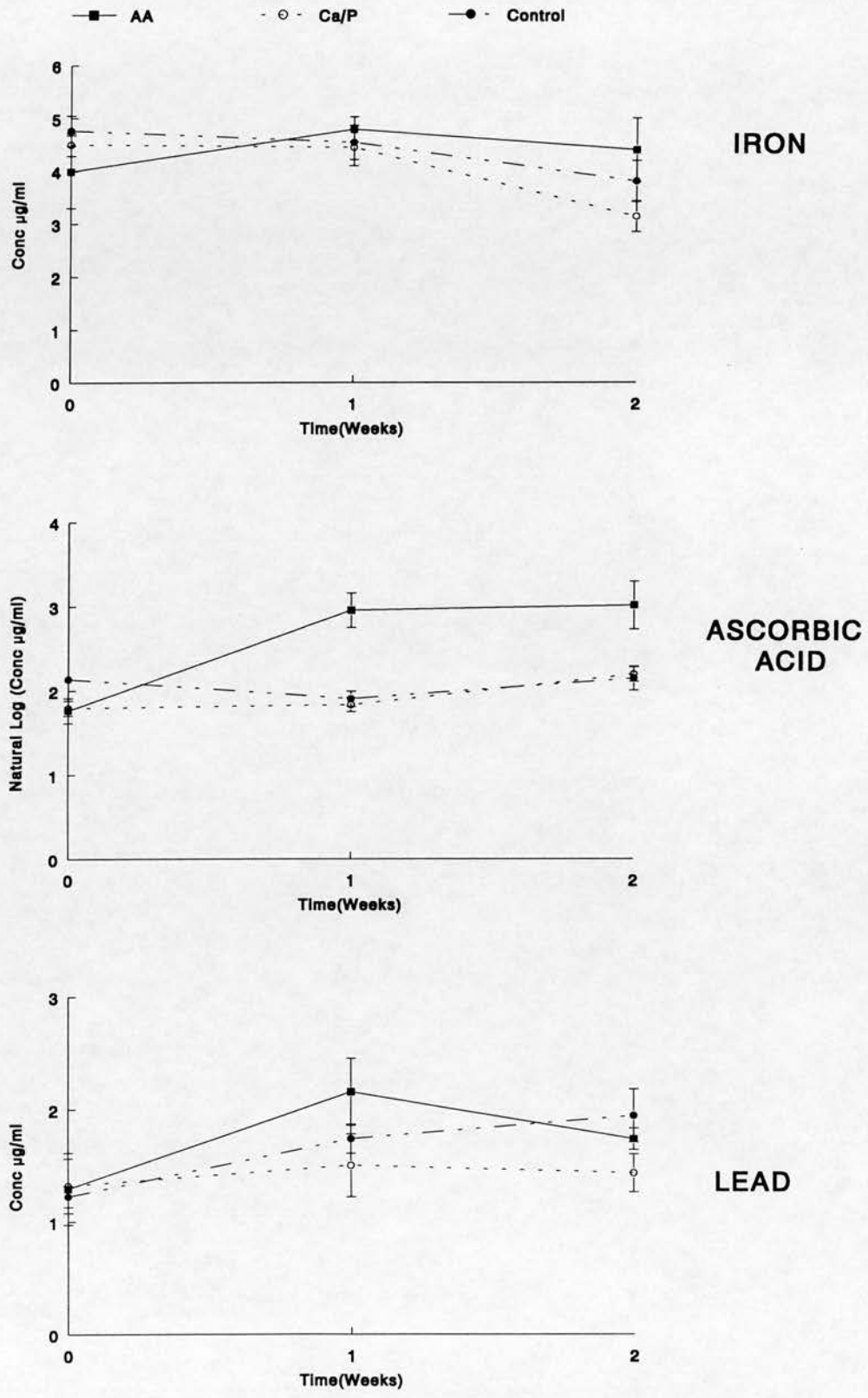


Figure 18. Effects of treating Wanlockhead lambs for two weeks with Calcium and Phosphorus (Ca/P) or Ascorbic Acid (AA) supplement on blood lead, plasma AA and plasma iron concentrations (Means ±SE, n=8).

Plasma ascorbic acid levels were more than three times higher in lambs treated with ascorbic acid after 1 ($p<0.001$) and 2 ($p<0.01$) weeks treatment, than in those fed calcium/phosphorus or those acting as controls. Plasma iron remained unaffected by the treatments.

3.3.8 Relationships Between Indices Of Mineral Status In Ewes And Lambs

Correlation coefficients were calculated for relationships within farms in the overall animal data. A significant positive relationship was found between ewe blood lead and copper ($r=0.52$:$p<0.05$) in Wanlockhead, but not in Moniaive. There was no association between lead in ewe's milk and lamb blood at any time on either farm, nor for lead in blood and milk in ewes from the two farms. Furthermore, no significant relationship was established between lead in ewe and lamb blood at any given age in either area.

Correlation coefficients between blood lead and other blood parameters in lambs of different ages from both farms are displayed in Table 30. At 1 week of age a significant positive correlation was evident between blood lead and copper (0.59, $p<0.01$), and similarly at 8 weeks of age between blood lead and zinc (0.50, $p<0.05$) on the control farm. At Wanlockhead, a positive correlation of 0.64 ($p<0.01$) was apparent between lead and zinc in 4 week old lambs.

Further statistical analysis of the consistency of blood lead values in individual lambs at Wanlockhead gave the following significant positive correlations: 0.82 ($p<0.01$) for lambs aged 1 and 4 weeks; 0.68 ($p<0.01$) at 4 and 8 weeks; 0.84 ($p<0.001$) at 4 and 12 weeks; and 0.90 ($p<0.001$) for lambs at 8 and 12 weeks.

TABLE 30

Correlation coefficients for blood lead with other blood factors in lambs of different ages within the two farms.

AGE (Weeks)		n	COPPER	ZINC	PHOSPHORUS	CALCIUM	ASCORBIC ACID
1	Wanlockhead	24	0.10	-0.14	-0.16	0.11	-
	Moniaive	18	0.59**	-0.43	-0.08	-0.32	-
4	Wanlockhead	23	0.11	0.64**	0.16	0.17	-
	Moniaive	18	-0.19	-0.32	-0.16	0.15	-
8	Wanlockhead	18	-0.26	-0.26	-0.02	0.18	-0.33
	Moniaive	18	-0.43	0.50*	0.05	0.21	0.21
12	Wanlockhead	12	-0.21	-0.01	0.54	-0.53	-
	Moniaive	18	0.16	-0.14	0.40	-0.32	-

Note: $^{*}p<0.05$ and $^{**}p<0.01$.

Correlations between blood lead and visible signs of the locomotor disorder and/or death were not significant at any age in Wanlockhead lambs.

Table 31 presents the correlation analysis carried out on lamb blood lead, ascorbic acid and iron in lambs of varying age fed different dietary supplements. Significant negative correlations were found in Group 1 between lead and ascorbic acid prior to, and after 1 weeks treatment with calcium/phosphorus ($p<0.05$). Blood lead was also correlated negatively with plasma ascorbic acid after two weeks treatment of the vitamin (Group 2: $p<0.05$). Positive correlations were common between lead and iron and significant ($p<0.05$) for Group 1 after one week and for the final samples from unsupplemented lambs. Finally, a negative correlation was apparent ($p<0.01$) between ascorbic acid and iron in lambs fed ascorbic acid for two weeks.

3.3.9 Reproducibility Of Laboratory Estimations

As previously outlined, a number of blood and milk samples were run as 'blind duplicates' as a check on reproducibility of laboratory results in the animal studies. The following coefficients of variation (CV) (SD/mean x 100%) were established for the blood analyses in Experiment 1 : lead (n=15), 8.4%; zinc (n=12), 7.6%; copper (n=14), 6.8%; calcium (n=15), 5.5%; and phosphorus, (n=15) 6.0%. Ascorbic acid 'blind duplicates' were run for both leucocyte and plasma determinations with one in four samples tested, numbering 9 and 10 samples, respectively. The C.V. for leucocyte AA was unacceptably high (22.8%), and the results were discounted.

TABLE 31

Correlation coefficients between blood lead (Pb), plasma ascorbic acid (AA) and plasma iron (Fe), according to age and treatment of Wanlockhead lambs.

AGE (Weeks)	TREATMENT	Pb versus AA n		Pb versus Fe n		AA versus Fe n	
1	-	8	-0.71*	8	-0.49	8	0.65
	-	8	0.52	7	0.44	7	0.42
	-	8	-0.05	8	0.61	8	-0.18
2	CALCIUM/PHOSPHORUS	8	-0.82*	8	0.76*	8	-0.54
	ASCORBIC ACID	6	0.70	8	0.58	6	0.20
	CONTROL	8	-0.27	8	0.67	8	-0.51
3	CALCIUM/PHOSPHORUS	8	0.33	8	0.33	8	-0.13
	ASCORBIC ACID	6	-0.82*	6	0.78	6	-0.95**
	CONTROL	7	0.04	7	0.84*	7	-0.18

Note: *$p<0.05$ and **$p<0.01$.

The C.V. for plasma AA on the other hand was 9.8%. A C.V. of 12.8% was obtained for 28 blind duplicate ewe milk samples, while differences between one in five duplicates for herbage, soil and tissue analyses was always under 10%. Finally, C.V.'s for the Experiment 2 analyses were 4.2% for blood lead (six samples), 4.8% for plasma AA (13 samples) and 5.8% for plasma iron (6 samples). With the exception of leucocyte AA determinations, the analytical results are reliable. Results for reference materials showed differences of less than 10%.

3.4 DISCUSSION

The overall purpose of this study was to reopen an inconclusive enquiry implemented over thirty years ago into the effects of past lead mining activity on young lambs (Butler, Nisbet and Robertson, 1957). Surprisingly, deaths in young lambs from the locomotor disorder are almost as severe today as then, the degree of environmental pollution is still high and farmers have learnt no lessons. The present work included an in depth examination of ewe and sequential lamb blood samples over the whole time period when deaths occur, as well as analysis of organs from lamb casualties, soils and herbage.

Before getting down to detailed discussions, the relationship of the present results in Wanlockhead and on the control farm of Moniaive to those of previous works are established. The limitations of a single control comparison are brought to the reader's attention, given that the normal range for all measured parameters is quite large and therefore differences between farms are likely to occur for reasons other than pollution.

3.4.1 Mineral And Vitamin Concentrations In Blood

Lead

The blood lead result for ewes in Moniaive was 'normal' whereas the result for ewes in Wanlockhead was almost double the recognised upper normal value, with a geometric mean of 0.42 μgml^{-1} Pb (Table 20). The normal level of lead in sheep blood is between 0.05 and 0.25 μgml^{-1} Pb (Donovan et al, 1969; Kaneko, 1980). For example, Allcroft (1950) has shown that the normal mean concentration of lead in sheep blood is 0.139 +/- 0.01 μgml^{-1}. Low mean lead levels in sheep from three areas of the Nile delta region of Egypt were 0.06, 0.07 and 0.08 μgml^{-1} (Takla et al, 1989). Other research for ewes grazing land contaminated by past mining activity vary in their results. Allcroft and Blaxter (1950) reported a mean blood lead concentration of 0.20 μgml^{-1} for ewes aged from one to six years grazing in Derbyshire near old lead spoil heaps; no significant correlation was established between the age of sheep and the concentration of lead in blood. Stewart and Allcroft (1956) reported a mean ewe blood lead of 0.55 μgml^{-1} for samples taken in the summer and autumn from a similar site in the Pennines. Mean lead values obtained in the following October and February were 0.36 μgml^{-1} and 0.35 μgml^{-1} lead, respectively, on one farm and 0.23 μgml^{-1} in February for another. The ages of the sheep in these studies were not reported.

Mean blood lead values obtained in July from two year old sheep on an adjacent farm to that in Wanlockhead had a mean lead value of 0.67 μgml^{-1} (Moffat, 1982). The present study area would appear to have among the highest reported ewe lead levels in blood from such areas. It is difficult to explain why, given omissions of detail in some studies concerning the age of animals, the degree of soil and pasture pollution as well as the actual time of year samples were taken. The last of these is perhaps of

most importance since soil ingestion probably represents an important pathway for entry of lead into the body and will vary with the time of year, weather factors such as rainfall, type of pasture and its grazing management. These points also relate to comparisons of blood and tissue measurements of other elements in the present study.

Normal blood lead levels in lambs have been described as less than 0.25 μgml^{-1} by Butler et al. (1957), being largely similar to the range found in ewes. The current study suggests that there may be a seasonal trend in normal lambs. In Moniaive, normal lamb values fell from 0.23 μgml^{-1} Pb at four weeks of age to 0.15 μgml^{-1} Pb at 8 weeks. Furthermore, the seasonal trend was amplified in Wanlockhead lambs, in which values peaked at 4 weeks of age with a mean of 1.45 μgml^{-1} Pb declining at 12 weeks of age to 0.67 μgml^{-1} Pb (Figure 13 and Appendix 1). Recent work at Moredun Research Institute has confirmed elevated lead concentrations in newborn lambs (N.F. Suttle, personal communication, 1992).

In a study of the same area some 30 years ago Butler et al. (1957) found blood lead levels ranging between 0.03 μgml^{-1} Pb and 2.5 μgml^{-1} Pb (mean 0.70 μgml^{-1} Pb), although the age of lambs on sampling is not reported. Such lead levels in lambs are not unduly high when compared to those from studies in other lead contaminated areas. Stewart and Allcroft (1956) reported mean blood lead levels of 1.44 μgml^{-1} (0.54-2.70 μgml^{-1}), while Clegg and Rylands (1966) described a mean blood lead value of 1.39 μgml^{-1} (0.85-2.40) in young lambs grazing land in former mining areas. The first of these studies reports mean blood leads taken in the summer and autumn from clinically affected lambs; the second is the mean blood lead for four affected lambs which died during May and June.

The values recorded in affected lambs in Wanlockhead both past and present show that the lead content of blood varies over a wide range and that, in many instances but not in all (Butler, Nisbet & Robertston, 1957) it was abnormally high.

Copper

Ewe plasma copper concentrations of 0.88 μgml^{-1} and 0.61 μgml^{-1} were found in Moniaive and Wanlockhead farms, respectively (Table 20). Stewart and Allcroft (1956) in their study found a mean of 0.72 μgml^{-1} (0.50-1.00) in clinically normal ewes. Only a slight variation exists in reported ranges of normal copper concentration in sheep plasma/serum. For example, Doxey (1977) gave a normal range for serum copper of 0.60-1.20 μgml^{-1} while Kaneko (1980) and Blood and Henderson (1974) reported serum ranges of between 0.58-1.60 and 0.70-1.30 μgml^{-1}, respectively. On this basis plasma copper in ewes from Moniaive are normal, while some values in Wanlockhead are deficient. The difference between farms could however reflect differences in copper supply that have nothing to do with mining activity.

The mean plasma copper levels for lambs studied over the twelve week sampling period in Moniaive and Wanlockhead are largely similar with a mean of 0.75 and 0.71 μgml^{-1}, respectively, (Appendix 1). Serum copper values reported for clinically affected lambs showing signs of a locomotor disorder in the Pennines had a mean of 0.74 μgml^{-1}, and a range of between 0.30 and 1.30 μgml^{-1} (Stewart and Allcroft, 1956). Clegg and Rylands (1966) quoted values of 2.20, 0.80 and 1.00 μgml^{-1} in three affected lambs in North Derbyshire.

Zinc

Mean plasma zinc concentrations in both ewe flocks were

slightly below the normal range (Table 20). Normal plasma zinc in sheep has been reported as being between 0.70 and 1.50 μgml^{-1} (Moredun Research Institute, 1990). Mean plasma zinc levels collected from thirty 2.5 year old ewes throughout a year were 0.74 μgml^{-1} and thought to be almost adequate for optimal animal production (Yu et al, 1987).

Similar and normal mean values were found in lambs in Wanlockhead (0.82 μgml^{-1} Zn) and in Moniaive (0.81 μgml^{-1} Zn) over the twelve week study (Appendix 1). These findings are in stark contrast to those reported by Butler et al. (1957) who found that lead exposed lambs had a mean blood zinc of 3.69 μgml^{-1} (2.79-5.31), while unexposed lambs had a mean of 2.03 μgml^{-1} (1.54-2.58). Both groups had unusually large concentrations of zinc which may indicate the measurement of whole blood compared to plasma concentrations in the present work. However, while the difference between groups in the study over thirty years ago may be real, it is possible that collection and laboratory techniques were not as stringent as today and that all samples were contaminated. For example, it is now known that the type of container or bung used in the blood sample process may influence the result of zinc analyses (Parker, 1972). Alternatively, the zinc contamination risk in Wanlockhead may genuinely have receded over the thirty years, but Butler, Nisbet and Robertson, 1957 do not quote herbage/soil zinc levels which could be compared with present samples.

Phosphorus

Levels of phosphorus in the plasma of ewes do not differ significantly from each other and fall well within the normal range, with a mean of 1.58 $mmoll^{-1}$ in Wanlockhead and 1.77 $mmoll^{-1}$ in Moniaive (Table 20) despite the fact that published inorganic phosphorus ranges for sheep

plasma differ substantially at the lower end. Normal ranges of 1.61 $mmoll^{-1}$ to 2.36 $mmoll^{-1}$ (Kaneko, 1980); 0.90 to 2.55 $mmoll^{-1}$ (Doxey, 1977); and 1.30 to 2.25 $mmoll^{-1}$ (Blood et al, 1983) are all cited.

According to Kaneko (1980), young animals usually have a higher and more variable concentration of phosphorus than older animals. Not unexpectedly therefore the mean blood levels over the twelve week period are significantly higher in lambs compared to their dams in the present study, averaging 2.78 $mmoll^{-1}$ in Wanlockhead, and 2.81 $mmoll^{-1}$ in Moniaive (Appendix 1). Lambs on the former farm had a mean value well above the normal limit at 4 weeks of age. Clegg and Rylands (1966) also quoted high phosphorus levels of 3.23 and 3.16 $mmoll^{-1}$ in two out of three young lambs affected by a locomotor disorder, while Butler et al. (1957) could find no difference between affected and normal lambs.

Calcium

Results for ewe plasma calcium in both areas are normal with a mean value of 2.14 $mmoll^{-1}$ in each farm area (Table 20). Similar ranges of calcium in sheep plasma are reported by Kaneko (1980) and Blood et al. (1983) of 2.87 $mmoll^{-1}$ to 3.19 $mmoll^{-1}$ and 2.86 $mmoll^{-1}$, to 3.24 $mmoll^{-1}$, respectively. On the other hand a normal range of 2.10 $mmoll^{-1}$ to 2.80 $mmoll^{-1}$ has been reported by Doxey (1977).

In lambs, plasma calcium levels are generally higher than in ewes but still fall within the same normal ranges, with similar overall means of 2.66 $mmoll^{-1}$ and 2.74 $mmoll^{-1}$ over twelve weeks for the Wanlockhead and Moniaive farms, respectively, (Appendix 1). Clegg and Rylands (1966) reported three calcium levels in affected lambs as 1.62, 2.62 and 2.44 $mmoll^{-1}$ in Derbyshire (i.e. one deficient value), while Butler et al. (1957) concluded that the calcium level in the majority of affected lambs did not

differ significantly from those for a normal group of lambs.

Ascorbic Acid

Published values of plasma ascorbic acid concentrations in sheep are not widely reported. Mean levels of 8.1 μgml^{-1} and 10.9 μgml^{-1} in eight week old lambs in Wanlockhead and Moniaive, respectively, were significantly different ($p<0.05$). Eighteen-month-old sheep grazing on pasture and fed corn, bran, mineral, and trace element supplements had a mean serum ascorbic acid level of 13.0 μgml^{-1} (Squibb et al, 1953). MacPherson (1984) found mean plasma ascorbic acid concentrations ranging from 4 to 8 μgml^{-1} in clinically normal wether sheep and individual values ranging from 1.1 to 9.3 μgml^{-1}.

3.4.2 Lead Concentrations In Ewe Milk

The geometric mean lead result of 0.14 μgml^{-1} in milk from the Wanlockhead ewes (Table 22) is similar to reported figures in other contaminated areas. Five ewes known to be grazing in the vicinity of former lead mining activity in the Pennines were found to have lead concentrations of 0.13, 0.10, 0.07, 0.09 and 0.11 μgml^{-1} in their milk (Stewart and Allcroft, 1956). Quarterman (1986) reported that concentrations of lead in milk are normally </= 0.02 μgml^{-1}, a figure close to the geometric mean of 0.03 μgml^{-1} found in Moniaive ewes. Generally speaking, data for lead in sheep milk are scarce although corresponding data for cow milk are numerous. A normal lead range has been reported as 0.028 to 0.030 μgml^{-1} for cow milk, being noticeably lower than the mean of 2.26 μgml^{-1} in milk taken from lead-poisoned cattle (Ineson, 1983). Other cows fed silage containing approximately 300 μgg^{-1} lead per kilogram dry matter produced milk with up to 0.14

μgml^{-1} lead (Quarterman, 1986).

3.4.3 Mineral Concentrations In Lamb Tissue

Liver Lead

The geometric mean liver lead found at Moniaive (8.6 μgg^{-1}) is typical of uncontaminated areas. Normal liver lead levels have been reported as less than 10 μgg^{-1} (Butler et al, 1957; and Stewart and Allcroft, 1956), with normal background levels of 2.2 μgg^{-1} (Humphreys, 1991) and 1.48 μgg^{-1} (Schulz-Schroeder, 1991) reported. The geometric mean value of 56 (35-89) μgg^{-1} in Wanlockhead can be compared with those found in other former lead mining areas where lambs showing clinical symptoms of a locomotor disorder had higher liver lead values of 54 to 376 μgg^{-1} (Stewart and Allcroft, 1956) and 48 to 400 μgg^{-1} lead (Clegg and Rylands, 1966). The work carried out by Butler et al. (1957) in Wanlockhead produced a similar lead mean to that found in the present study. The principal accumulation of lead occurs early in life (see blood leads) so the values in the liver may fall as the liver, like the lamb, grows in size. The age of lambs in the other studies is unknown, but they may have been younger casualties.

Liver Copper

Normal copper levels in the liver show a particularly wide range because this is the storage site for the element. The norm is reported as >20 μgg^{-1} for sheep but values of 100 to 300 μgg^{-1} are common (N. Suttle, 1992, personal communication). Copper liver concentrations of between 22 and 161 μgg^{-1} were found in Australian lambs from a Saudi Arabian slaughter house (Sadiq and Alam, 1991). The present control geometric mean of 163 μgg^{-1} reflects a generous provision of copper from ewe to

offspring (Suttle, 1986). On the other hand, results for Wanlockhead lambs with a geometric mean of 19 μgg^{-1} copper are marginally subnormal, and lower than values from another lead mining area, where a range of 20 to 80 μgg^{-1} was found (Clegg and Rylands, 1966). Butler et al. (1956) did not report values for liver copper. The low copper status at Wanlockhead could be explained in terms of copper supply : Clegg and Rylands (1966) did not report herbage copper. Alternatively there may be a lead or zinc interference in copper metabolism. These points are discussed later.

Liver Zinc

Mean zinc concentrations in livers of lamb casualties were essentially similar at 166 μgg^{-1} and 204 μgg^{-1} on the Wanlockhead and Moniaive farms, respectively, and consistent with values reported from Wanlockhead in 1957 by Butler et al. (172 : 85-430 μgg^{-1}) who gave a normal zinc value of 93 (62-118) μgg^{-1}. Normal mean zinc values for control sheep were reported as 126, 141, and 125 μgg^{-1} by Bremner et al. (1976). A high mean zinc concentration of 1167 μgg^{-1} was found in the livers of seven sheep aged 18-24 months which were fed daily with a substance emitted from a copper and zinc factory (Bires et al, 1991).

Kidney Lead

Like liver levels, the lead levels in kidneys are distinctly increased in lambs from old lead mining areas compared with normal lambs. Thus in Wanlockhead, a geometric mean value of 87.6 μgg^{-1} can be compared with a range of between 30 and 500 μgg^{-1} in North Derbyshire, and 27 and 715 μgg^{-1} in the Pennines (Clegg and Rylands, 1966; Stewart and Allcroft, 1956). Butler et al, (1957) reported a similar mean value (77 μgg^{-1}) to the present in

Wanlockhead with a range of 11-506 μgg^{-1}. Ineson (1983) reported values of 5 to >100 μgg^{-1} lead in kidneys from poisoned lambs.

Vastly different is the geometric mean of 1.8 μgg^{-1} lead in Moniaive lambs which corresponds to normal values quoted by Stewart and Allcroft, 1956 (8-10 μgg^{-1} Pb); Humphreys, 1991 (mean of 3.5 μgg^{-1} Pb); and Shulz Schroeder, 1991 (mean of 1.74 μgg^{-1} Pb).

Kidney Copper

Kidney copper concentrations are reduced in copper deficiency and increase during copper toxicity, but show little variation in response to copper intake between these extremes (Suttle, 1986). It is hardly surprising that there is no difference in kidney copper between the values of 13.7 μgg^{-1} in Wanlockhead and 13.1 μgg^{-1} in Moniaive. Bremner et al. (1976) reported kidney copper levels to be normally around 12 μgg^{-1}. Kidney copper values were unavailable from other former lead mining areas.

Kidney Zinc

Kidney zinc levels of 101.6 μgg^{-1} and 85.4 μgg^{-1} in Wanlockhead and Moniaive, respectively, can be compared to groups of six adult Black face ewes given one of three sewage sludge enriched top soils containing zinc concentrations of 6500 μgg^{-1}, 1700 μgg^{-1} and 2900 μgg^{-1} as a dietary supplement of 1.5% for the first 21 days, and 7.5% D.M. for the next 41 days. Resultant kidney zinc mean concentrations were 161 μgg^{-1}, 125 μgg^{-1} and 137 μgg^{-1}, respectively, for the above three groups. Sheep fed a matched untreated soil (120 μgg^{-1} Zn) had a mean kidney zinc level of 120 μgg^{-1} (Suttle et al, 1991).

These kidney zinc contents can be further compared with a

range of 2720 to 7943 μgg^{-1} in the kidney cortices of lambs receiving a sole protein source of yeast contaminated with 840 μgg^{-1} zinc D.M. over five weeks and lambs maintained on a milk diet with kidney cortex zinc contents of 299 and 320 μgg^{-1} (Davies et al, 1977).

Bone Lead

Of the heavy metals measured in bone, lead once again emerges as the prime factor of contrast between farms. The geometric mean value of 191 μgg^{-1} for Wanlockhead is consonant with levels of 140 to 1000 μgg^{-1} reported in lead-poisoned lambs (Ineson, 1983) and values of 184 to 316 μgg^{-1} found by Stewart and Allcroft (1956) in the Pennines. The elevation of lead in bone on these farms can be guaged by comparison with a geometric mean of 13 μgg^{-1} found in Moniaive, and the maximum normal value of 20 μgg^{-1} reported by Butler et al, 1957.

Bone Copper

Copper levels in bone from affected and normal lambs are congruous, with means of 4.1 μgg^{-1} and 4.7 μgg^{-1}, respectively, from the Wanlockhead and Moniaive farms. There is little information on the normal copper concentration in sheep bone, but Suttle and Angus (1978) reported values of 3.5-4.6 μgg^{-1} in fat free bone in cattle with no effect of copper deficiency.

Bone Zinc

Similar mean zinc levels in bone of 100 μgg^{-1} and 101 μgg^{-1} in Wanlockhead and Moniaive lambs, respectively, can be compared to those in affected lambs in Wanlockhead (114 μgg^{-1}) and in normal lambs (84 μgg^{-1}) some thirty years ago (Butler et al, 1957). Results for both liver and bone zinc provide no evidence of zinc contamination.

Bone Phosphorus

Mean phosphorus concentrations in dry bone from the present study farms show no significant difference with a mean of 9.6% and 9.1% in Wanlockhead and Moniaive, respectively, depicting no abnormality. Essentially similar values were found for dry, fat-free bone in affected and normal lambs by Butler et al, (1957) with phosphorus means of 10.3% (9.2-11.7) and 9.8% (9.0-11.5), respectively, in their study of the same area. Reverton et al. (1974) reported two normal values for lambs of 10.6% and 10.5% and two low values of 8.6% and 8.0% in dry fat-free bone.

Bone Calcium

Due to laboratory error, bone calcium concentrations are unavailable for Wanlockhead and Moniaive lambs. Butler et al. (1957) reported values of 19.3% (17.1-22.8) and 19.4% (16.3-21.6) in dry fat-free bone for affected and normal lambs, respectively, in their study of Wanlockhead. Reverton et al. (1974) found normal levels of 21.9% and 20.4% calcium and low levels of 16.7% and 17.4% calcium in the dry fat-free bone of two lamb groups.

3.4.4 Mineral Concentrations In Soils

Lead

Normally total lead levels in farm soils range between 10 and 150 μgg^{-1} (Thornton, 1983), have a geometric mean of 40 μgg^{-1} (Archer and Hodgson, 1987) and a typical rural upper threshold value of 100 μgg^{-1} (East of Scotland College of Agriculture, 1988). The normal geometric mean lead level of 81 μgg^{-1} (43-207) in Moniaive is strikingly different from that of 3826 μgg^{-1} (1422-9540) in Wanlockhead. This latter value is similar to the mean

values of 2942 μgg^{-1} for a 'high' lead area in Derbyshire (Thornton and Abrahams, 1983) and 1653 μgg^{-1} (19-2976) reported from the contaminated Ystwyth Valley (Alloway and Davies, 1971). Samples of soil found near old smelting mills and ore washing tanks in the Wanlockhead area contained 19,500 and 17,800 $\mu gPbg^{-1}$, being an area with a particularly high incidence of the lamb locomotor disorder (Butler et al, 1957). Values on the Wanlockhead farm were slightly lower than those found in garden soils in the area (Table 5). It is clear from the heterogeneous dispersal of mining waste over the hillsides that there are likely to be massive variations in soil lead as well as other elements, even within fields. There may also be differences in lead concentrations with the depth of soil sample taken.

Copper

Total soil copper in Moniaive has a geometric mean value of 9 μgg^{-1} (8-10) which is normal when judged by standards of 20 μgg^{-1} (Purves, 1985; East of Scotland College of Agriculture, 1988), 18 to 22 μgg^{-1} (Blood and Henderson, 1974) and 2 to 60 μgg^{-1} (Thornton, 1983). The elevated geometric mean copper concentration of 91 μgg^{-1} (14-198) in soils from Wanlockhead is higher than the mean of 39 μgg^{-1} reported from a 'high' lead area in Derbyshire (Thornton and Abrahams, 1983). A European Economic Directive (86/278/EEC, 12.6.86) recommends that the threshold for total copper in soil should be in the range 50 to 100 μgg^{-1}.

Zinc

Normal total zinc concentrations in soils are similar to the geometric mean of 70 μgg^{-1} (61-77) found at Moniaive. For example, Thornton and Abrahams (1983) reported a mean zinc value of 87 μgg^{-1} in a low lead area of Derbyshire,

while Thornton (1983) described a range of 25-200 μgg^{-1} as normal. The East of Scotland College of Agriculture (1988) reported a typical rural value as 70 μgg^{-1} and an upper threshold value of 150 μgg^{-1} for total zinc in soils. The present geometric mean value (and range) of 326 μgg^{-1} (38-610) found in Wanlockhead is clear evidence of contamination and resembles levels found in other metal-rich soils. Alloway and Davies (1971) described a mean of 604 μgg^{-1} (182-1750) in the Ystwyth Valley, while a mean of 287 μgg^{-1} was given by Thornton and Abrahams (1983) for high lead areas in Derbyshire.

Phosphorus

Equivalent available phosphorus means of 2.2 μgg^{-1} and 2.3 μgg^{-1} in Wanlockhead and Moniaive can both be considered as low. Normal ranges have been reported as 10-50 μgg^{-1} (A.D.A.S, 1973) and as 26-75 μgg^{-1} (East of Scotland College of Agriculture, 1988).

Calcium

Although Wanlockhead had a significantly lower available calcium mean (48 μgg^{-1}) than the control farm (204 μgg^{-1}) both concentrations are well below normal ranges (500-2000 μgg^{-1}, Bradshaw and Chadwick, 1980; 1000-3000 μgg^{-1}, Mitchell, 1948).

Sulphur

Although no deficiencies in available sulphur are evident on the two farms, the Wanlockhead geometric mean of 12 μgg^{-1} is significantly lower than the value of 21 μgg^{-1} found in Moniaive. Sulphur concentrations of between 8-12 μgg^{-1} have been reported as normal (Chaney and Kershaw, 1986).

3.4.5 Mineral Concentrations In Herbage

Lead

The lead concentration in uncontaminated pastures is normally 0.3-1.5 μgg^{-1} (Mitchell and Reith, 1967) and rarely exceeds 2 or 3 μgg^{-1} (Underwood, 1977; Purves, 1985). Thus the geometric mean value of 2.6 μgg^{-1} (1.8-3.6) in Moniaive is normal. The geometric mean Wanlockhead value of 42.7 μgg^{-1} (14.5-97.9) is far lower than the mean of 278 μgg^{-1} reported by Butler et al. (1957) from the same locality. Samples in the present study were collected from all grazing areas, while those reported by Butler et al. (1957) were collected close to old smelting mills where the highest incidence of the locomotor disorder was found. Pasture in other former mining areas had a mean lead concentration of 427 μgg^{-1} (Stewart and Allcroft, 1956) and a range of 30-94 μgg^{-1} (Allcroft and Blaxter, 1950).

Copper

Herbage copper concentrations, like soils, are significantly higher in Wanlockhead compared to Moniaive with mean values of 13.6 μgg^{-1} (13.0-14.9) and 11.6 μgg^{-1} (10.2-13.1), respectively, ($p<0.05$). A mean herbage copper of 9 μgg^{-1} (2-15) was reported for permanent leafy grass samples from England and Wales (ADAS, 1975), while Suttle (1983) gave a mean for pasture samples submitted to Scottish Agriculture Colleges as 6.5 μgg^{-1} in summer and 8.5 $\mu gCug^{-1}$ in autumn.

Zinc

Results for herbage zinc concentration on the two farms are closer than the corresponding soil levels. Moniaive has a mean of 41.3 μgg^{-1} (28.0-59.5) and Wanlockhead 66.1

μgg^{-1} (28.9-126.5). The latter value is considerably lower than the mean level of 140 μgg^{-1} zinc (66-350) found in the Ystwyth Valley (Alloway and Davies, 1971). The mean zinc concentration in normal herbage in the UK is 51 μgg^{-1} (range 20-60; ADAS, 1975).

Phosphorus

Total mean phosphorus values for pasture of 2200 μgg^{-1} in Wanlockhead and 1500 μgg^{-1} in Moniaive are both at the lower end of the normal range. The mean phosphorus concentration for spring grass from 21 sites on improved Scottish hill pastures in 1985 was 4200 μgg^{-1} (N. Suttle and others, 1991, personal communication). Samples of permanent leafy grass analysed by ADAS (1975) since 1967 contained 3900 μgPg^{-1} (range 1500-4500 μgg^{-1}).

Calcium

Like phosphorus, total mean herbage calcium concentrations in both Wanlockhead (3500 μgg^{-1}) and particularly in Moniaive (2400 μgg^{-1}) are at the lower end of normal ranges. ADAS (1975) reported a mean of 5900 μgg^{-1} (3000-10,000), while a mean value from improved Scottish hill pastures sampled in 1985 was found to be 6200 μgg^{-1} (N. Suttle and others, 1991, personal communication).

Sulphur

Total mean sulphur concentrations are normal on both farms with a mean of 2000 μgg^{-1} (1620-2520) and 2100 μgg^{-1} (480-2760) in Wanlockhead and Moniaive, respectively. These are equivalent to mean sulphur results reported by ADAS (1975) of 1500 μgg^{-1} (1000-3500).

3.4.6 Pathogenesis Of The Locomotor Disorder At Wanlockhead

Lead

Previous research into the essentially similar locomotor condition in young lambs grazing former lead mining areas of Great Britain has not found a clear association with lead (Butler et al, 1957; Stewart and Allcroft, 1956; and Clegg and Rylands, 1966).

The present results strengthen evidence for an association between lead exposure and death rate in lambs in Wanlockhead by relating clinical signs and blood lead concentrations. Despite a mean blood lead of 1.09 μgml^{-1} in lambs approximately one week of age only one lamb showed evidence of any form of locomotor disorder even after being driven for some distance. Data shown in Table 24 indicate a significant ($p<0.05$) difference in blood lead in four week old lambs separated according to those exhibiting normal locomotion and those showing signs of the disorder. At eight and twelve weeks of age, lambs showing signs of the locomotor disorder have slightly elevated blood leads compared to unaffected lambs. Relating blood lead levels at one and four weeks, to the development of the locomotor disorder by eight and twelve weeks when most deaths occur, indicated a better predictive than diagnostic role (Table 25).

The fact that peak death rates occur about one month after peak concentrations of lead in blood may arise because lead is a cumulative poison which affects bone, while blood lead probably reflects lead 'in transit'. The peak rate of accumulation of lead in bone probably occurs when blood lead is at its maximum but the total lead in bone needed to give the locomotor disorder may only be reached when the blood lead peak has passed. Bone growth is still continuing at a time when lead may be hard to

shift.

It is of note that by twelve weeks of age the mean lamb blood lead of 0.67 μgml^{-1} in Wanlockhead (Figure 13) is closer to that of the ewe (Table 20) than at any other period ($p<0.01$, compared to $p<0.001$ at all earlier ages). Despite a raised blood lead, ewes from this area live and reproduce successfully and never show signs of a locomotor disorder. Lambs which survive to 12 weeks can be raised successfully but many who have shown signs of the locomotor disorder are slow to develop and are not worthy of sale in the autumn.

The cessation of affected cases by the age of twelve weeks is further backed by local knowledge where if a farmer can remove lambs at birth to unaffected pasture for around eight to ten weeks, he is very unlikely to lose any lambs from the locomotor disorder on their return to the former lead mining area.

Comparisons can be drawn between the present lead concentrations in blood from lambs on contaminated ground and limited work by Stewart and Allcroft (1956) from former lead mining areas in the Pennines. The lamb offspring of two groups of six and four ewes were blood sampled after parturition and thereafter at weekly intervals for five weeks. Blood lead levels of lambs at birth were very similar to those of their mothers (around 0.50 μgml^{-1}), but rose quite rapidly within three to five weeks to concentrations double those of their dams. Values in lambs at three weeks on the two farms were 0.95 μgml^{-1} and 1.40 μgml^{-1} lead, reaching a value of 1.20 μgml^{-1} by five weeks of age. These figures are clearly comparable with the present study of ewes and lambs (Table 20 and Figure 13.). All but two of the selected lambs remained healthy and free from detectable lameness up to the age of five weeks in the Pennines study. Assuming ten lambs were studied, then 20% of lambs were

affected by the locomotor disorder by the age of five weeks; this can be compared to signs of the disorder in 38% of lambs at four weeks of age in Wanlockhead.

It has been reported (e.g. Quarterman et al, 1977) that male sheep are more clinically susceptible to the toxic effects of lead than castrates and females, while Butler et al. (1957) reported that sixteen of the twenty affected lambs examined in Wanlockhead were male. All research to date on the locomotor disorder in young lambs in former lead mining areas (Stewart and Allcroft, 1956; Butler et al, 1957; and Clegg and Rylands, 1966) including the present, have found that the clinical disorder is confined to the months of May to July. It has been suggested by Quarterman et al. (1977) that this short period may coincide with the period between weaning and the disappearance of male hormones following castration, when lambs would still have the greater susceptibility potentially given by male hormones. In the present study, the male lambs were all castrated at two months of age. Current investigations suggest no relationship between blood lead and sex (Table 26). Furthermore, there is no significant difference between sexes in blood lead concentrations or the presence of the locomotor disorder.

This current investigation backs up previous studies showing that not all affected lambs have a high blood lead concentration (Butler et al, 1957; Stewart and Allcroft, 1956) but this does not eliminate lead as a major causative factor. Blood leads do not reflect cumulative exposure. Furthermore infrequent blood sampling may not have coincided with maximum lead exposure for each lamb, which is likely to vary from day to day as the ewe and lambs move about the fields and encounter different degrees of contamination. Bone disorders may by their very nature be slow to develop and a lag between maximal lead exposure and the appearance of

clinical symptoms is to be expected.

Copper

The differences in plasma copper values for lambs between the two farms at one and twelve weeks of age present a contrast (Figure 14). In the former case lambs in Moniaive have a lower plasma copper ($p<0.05$) than lambs in Wanlockhead, which is inconsistent with the high liver copper values in casualties on the control farm (Table 27). Lambs in Moniaive were caught and first sampled slightly sooner after birth than those in Wanlockhead. It may be that the rapid increase in plasma copper in the first 24 hours after birth could have lead to an age bias in the data (Howell et al, 1968). Plasma copper levels in one week old lambs from the control area were also significantly lower than their dams ($p<0.001$) but copper levels rise steadily thereafter on both farms. By twelve weeks of age, plasma copper levels in Wanlockhead are significantly lower than on the control farm ($p<0.01$). Wanlockhead ewes also have a significantly lower plasma copper value (Table 20), and lambs significantly lower liver copper values ($p<0.001$) than the controls (Table 27). Stewart and Allcroft (1956) also reported a few low blood copper values in Pennine ewes, but there was no evidence of a general hypocupraemia, such as is found where swayback in lambs occurs.

The reduction in body copper status in Wanlockhead occurs despite high soil copper content and a herbage copper concentration above normal. However soil ingestion can reduce the availability of dietary copper by over 50% (Suttle et al, 1975; Thornton and Abrahams, 1983). Soil ingestion may either be hindering the absorption of copper in the alimentary tract or be releasing a copper antagonist - in this instance probably lead and/or zinc.

Other research has suggested that increased lead levels

can reduce blood and liver concentrations of copper, whilst an increased copper intake may alter the effect of lead in the diet (Hemmingway et al, 1964; Petering, 1980). Such results imply a mutual metabolic antagonism which is not well understood, although lead toxicity may arise indirectly through interference with the availability and function of essential nutrients such as copper. Lead exposure may for example increase the turnover of body copper in anti-oxidant defence mechanisms, thus increasing the rate of copper depletion.

On an individual basis, one quarter of surviving lambs at one, four and twelve weeks have subnormal plasma copper levels below 0.60 $\mu g ml^{-1}$ in Wanlockhead. However the hypocupraemia is mild compared to that giving osteoporosis under experimental conditions (Suttle et al, 1972). Nevertheless, there may be an improvement in the clinical condition of Wanlockhead lambs from the provision of copper licks, since increased copper in the diet protects against the biochemical effects of lead as well as the metals toxicity (Petering, 1980). The requirement for copper and the impact of hypocupraemia on bone development might be increased during exposure to excess lead.

Zinc

A reference to Table 28 shows that despite five fold raised soil zinc levels in Wanlockhead, there is little influence on blood zinc levels over the twelve week period. Plant zinc concentrations (Table 29) in the two areas are however similar and imply either a low availability of soil zinc, plant control of uptake or a lead-zinc interaction which inhibits zinc uptake by herbage. The low pollution factor for zinc in herbage is relevant for spoil reclamation projects.

Accordingly, environmental zinc contamination is unlikely

to predispose the locomotor disorder, despite suggestions by Butler et al. (1957) to the contrary. Indeed, it has been reported that extra dietary zinc can act as a protective agent in lead exposure and reduce tissue lead levels (Cerklewski and Forbes, 1976; Petering, 1980), while diets low or deficient in zinc increase lead absorption and tissue lead concentrations (Cerklewski et al, 1976). Lead and zinc display a mutual metabolic antagonism which is likely to take place in the gastrointestinal tract. Zinc-copper-lead interactions may also occur (Klauder, 1975; Murphy et al, 1975). On this basis, higher environmental zinc may limit the incidence of the locomotor disability in lambs from Wanlockhead.

Lamb plasma zinc concentrations on both farms are consistently higher than those in their dams (Figure 15 and Table 20) : this may be explained by a high availability of milk zinc. This pattern is dissimilar to ewe and lamb lead levels where lambs in Moniaive at eight and twelve weeks showed no significant difference to ewe concentrations, despite being much higher ($p<0.001$) at one and four weeks. This probably reflects differences in availability of heavy metals once rumen function has fully developed. Although soluble zinc, like lead, should be converted to zinc sulphide in the rumen, the latter is acid soluble and zinc should leave the acid environment in an absorbable form.

Phosphorous and Calcium

Physiologically, the most important result may be the abnormally high plasma phosphorus concentrations seen in Wanlockhead lambs at four weeks of age, which may reflect impairment by lead of phosphorus uptake by the skeleton (Figure 16). The apparent fall in plasma phosphorus to levels below those in Moniaive lambs may be biased by the loss of most affected lambs from the Wanlockhead flock (25% by 8 weeks of age; 50% by 12 weeks of age). Lead-

induced changes in phosphorous and calcium metabolism and absorption have been reported, and may well explain some of the lower values in Wanlockhead lambs (e.g. Gruden, 1975). In a later discussion the inverse effects of low dietary levels of calcium and phosphorous are shown to be instrumental to high uptakes of lead.

Ascorbic Acid

Plasma ascorbic acid values were lower ($p<0.01$) for eight week old lambs in Wanlockhead than in Moniaive (Appendix 1). This is of interest since research has long suggested that ascorbic acid might ameliorate the condition of lead toxicity, the vitamin having both a therapeutic and a prophylactic value. For example, Holmes et al. (1939) suggested a clinical resemblance between scurvy and lead poisoning : they treated lead workers with 100mg of ascorbic acid daily and reported a clinical improvement. Marchmont-Robinson (1941) treated other workers exposed to lead with 50mg of ascorbic acid daily and also reported that workers felt better.

More recent research has provided conflicting results on the ability of ascorbic acid to reduce the availability of lead. It has been established that the toxicity's of for example, selenium, cobalt, vanadium, mercury and copper can be reversed by ascorbic acid but not the toxicity of lead (Hill, 1980). In work with chicks a factorial experiment examined the effect of ascorbic acid (0.5%), ferrous iron (1,000 ppm) and a combination of the two on the toxicity of 500 ppm lead given as the chloride. No interaction between ascorbic acid and lead was established, although iron reduced lead concentrations in the femur and kidney; ascorbic acid had no such effect on the femur and only a moderate effect on the kidney. A similar study with rats, in which iron and ascorbic acid were tested separately, found that the beneficial effects as far as lead toxicity was concerned

resided entirely with the iron (Suzuki and Yoshida, 1979). It may well be that these differences arise from the fact that rats and chicks, unlike humans, can synthesise their own ascorbic acid.

Turning to lambs, Butler et al. (1957) noted that both the gross and histological features of the disorder found at Wanlockhead were very similar to the osteoporotic condition produced by a mild deficiency of ascorbic acid in children. In Derbyshire, affected lambs were treated with ascorbic acid in view of the similarity of skeletal lesions of lead poisoning and of scurvy (Clegg and Rylands, 1966). All affected lambs were treated daily with 200mg or 800mg ascorbic acid for a total of four weeks. Within three or four days, all lambs were moving more freely and it was suggested that the Vitamin C requirements of the affected lambs had not been met. However it is currently noted that no control lambs were used in this study and that spontaneous reductions in lead status and improvements in clinical condition are a feature of the disorder.

A current investigation by at least one farmer in Derbyshire (Farmers Weekly, 1979; Atkin, 1985, personal communication) revealed the following. Through regular checks ailing lambs are identified early, treated with large doses of Vitamin C and, then immediately removed to uncontaminated ground where they generally recover. Lambs left on polluted ground following treatment did not improve and were likely to deteriorate further. Lambs in Leadhills and Wanlockhead which are removed from polluted ground in the early stages of the locomotor disorder and given no treatment also usually recover. Moreover ruminants synthesise their own ascorbic acid by around 2-3 weeks of age. In view of these uncertainties surrounding the effectiveness of ascorbic acid on lead toxicity, a directly controlled dietary supplement of ascorbic acid was administered to young lambs permanently

grazing polluted ground in the following year.

Relationships Between Mineral Concentrations In Lambs

In general, the correlation co-efficients displayed in Table 30 for blood lead and the various other blood factors measured in lambs of different ages from the two areas do little to explain the locomotor disorder. Lead and zinc concentrations positively correlate in four week old lambs in Wanlockhead ($p<0.01$) indicating signs of common pollution in the area.

Pathways for Lead Transfer from Environment to Animal

Soil Ingestion

Analytical data recorded in Tables 28 and 29 for soils and herbage illustrate the environmental contamination affecting the diet of sheep and lambs. Whilst it may be thought that herbage is the most important factor to be considered, it is now well established that soil is involuntarily ingested in large amounts by adult grazing sheep as a dietary contaminant. For example, it has been reported that up to 30% of the dry matter intake of sheep may be through soil ingestion (Thornton and Abrahams, 1983; Thornton, 1983; and Russell et al, 1985). This means that soil ingestion in the contaminated former mining area could represent the major route of metal intake. Ingestion of soil has been found to be highest in early spring when grass is in short supply, falling to a low in summer and rising again in the fall of the year (Field and Purves, 1964; Thornton and Abrahams, 1983) In addition to season, other factors including soil type, weather (especially the amount of rainfall), types of pasture and management factors will all play a part in determining the amount of soil ingested (Healy, 1969; Thornton, 1983). Pasture lead has been found to be less

important than soil lead as a pathway in both sheep and cattle (Blaxter, 1980; Thornton, 1983; and Davies and Thornton, 1988).

The question of soil ingestion by suckling lambs clearly needs to be addressed in former lead mining areas. Young lambs are known to be and indeed were observed to be inquisitive, often licking the surfaces of soil, spoil material and contamination on herbage around them. The total daily intake of soil lead for an individual lamb will of course be related to both the soil lead content and amounts ingested. In addition lead contamination from the udder of ewes lying on toxic ground and dust contaminated pastures is likely to be available to affect the lamb. This must clearly be an important route to a young lamb at a time when the absence of or poor development of a rumen enhances the uptake of heavy metals.

Placental Transfer

By around one week of age blood lead levels in lambs are already excessively elevated in Wanlockhead. This may partly be explained through placental transfer of lead from the ewe, given her abnormally high mean blood level of 0.42 μgml^{-1} but at this level it is unlikely that storage of harmful amounts of lead would occur in the foetuses.

An example of the amount of lead crossing the placenta from the maternal to the foetal circulation can be found in an experimental study where two groups of ewes were given finely powdered elemental lead sufficient to maintain blood lead levels of 0.34 μgml^{-1} and 0.18 μgml^{-1}, respectively, throughout gestation. The lambs from the two lead-exposed groups had blood lead levels of 0.25 μgml^{-1} and 0.17 μgml^{-1}, respectively, at between two and four weeks of age while control lambs had blood lead

levels of 0.06 μgml^{-1} (Carson et al, 1974).

Transfer via Milk

Another influence is that of drinking lead-contaminated milk from the ewe, results in Table 22 indicating a five fold increase in lead from Wanlockhead milk. Milk lead may be further added to during lactation through resorption of calcium from bone and a subsequent release of lead into the circulation when dietary supplies of calcium become inadequate. The outer layers of ewe bone are likely to be particularly high in lead in Wanlockhead following high winter soil ingestion and increased pasture lead ingestion. Although lead in milk per se is likely to contribute to body lead in Wanlockhead lambs, no correlations were established between lead concentrations in milk and lamb blood at any age in either area. If milk lead intake alone is important a larger proportion of lambs would be expected to be affected by the locomotor disorder given the consistently high lead levels in milk from Wanlockhead ewes. Nevertheless, recent work at the Moredun Research Institute has shown barely detectable increases in milk lead (less than 0.01 μgml^{-1}) in ewes fed contaminated topsoil can cause substantial increases in blood lead in the suckling lamb (up to 0.1μgml^{-1}): (N.F. Suttle, personal communication). It is also noted that lead in Wanlockhead ewe's milk is ten times the concentration found in the domestic water which was so influential for man.

In addition, milk can play a very important part in determining the amount of lead and other heavy metals absorbed from other sources by an animal. For example, it is known that the absorption of lead is very high in suckling animals, decreases rapidly at weaning and decreases more steadily thereafter. For example, adult rats given milk as their only food for three days

increased the absorption of a single dose of 203 Pb by more than fifty times, with smaller increases monitored for other heavy metals (Kello and Kostial, 1977). Quarterman (1986) also reported that the absorption of lead is very high (approx. 50%) in suckling animals, while milk-only diets given to rats dosed with a lead solution increased the carcass retention of lead (Quarterman and Morrison, 1981). Milk had a much smaller or an inhibitory effect on lead absorption when accompanied by solid food.

It is not fully known how soon after milk feeding that heavy metal absorption increases nor how long after milk feeders are returned to solid food that heavy metal absorption decreases. If these adaptation times are short, then it is likely that milk between meals could affect heavy metal absorption (Quarterman, 1981; Quarterman and Morrison, 1981) but results will clearly depend on the overall composition of the diet and the amount of milk consumed (Quarterman, 1986). Several milk constituents including lactose, fat, some low molecular weight ligands and lactoferrin may be responsible for enhancing lead absorption (Quarterman and Morrison, 1985; Bushnell and De Luca, 1981).

Lambs on the present study farms were probably on a completely milk diet up to the age of 2-3 weeks, serving to heighten the absorption of lead from the gut at a time when soil and spoil, as well as udder contamination are likely. High lead absorption is probably responsible for the rise in blood lead levels up to the age of four weeks (Figure 13). Thereafter it is probable that as the grass content of the diet increases lead absorption decreases. This is also a stage where the lamb is ageing, blood volume is expanding rapidly and the increasingly maturing gut is becoming more discriminating against heavy metal absorption (Momcilovic and Kostial, 1974; Quarterman and Morrison, 1978). By the time weaning approaches blood

lead levels have decreased close to ewe levels on each farm.

Drinking Water

Hill streams in Wanlockhead have been found to contain low lead concentrations of between 0.01 and 0.03 μgml^{-1}, while the main waterflow through the former mining area has a mean lead concentration of 0.25 μgml^{-1} (Moffat, 1984). Stream sediments of the Wanlock Water contain lead levels of up to 10,000 ugg^{-1} : if disturbed by the animal crossing or stepping in the water it is likely that involuntary ingestion of high amounts of lead may occur on drinking. Pools of water lying on spoil material must also contain high lead levels. The predominant fluid source of the lamb however is milk which has a lead concentration approximately half that of the contaminated water.

Transfer to the animal from other dietary constituents

The generally low level of macro nutrients in soils and herbage (Tables 28 and 29) may influence body lead status since research has shown that the lead toxicity is heavily influenced by the major mineral composition of the diet. Soil concentrations of phosphorus and calcium are especially low in the former lead mining area, with low levels of herbage phosphorus and calcium also evident.

Animal studies have shown that subnormal intakes of calcium and phosphorus can increase the retention of ingested lead in body tissues, while increased levels of dietary calcium and phosphorus can reduce lead absorption. For example, rats on a lead-supplemented diet deficient in calcium or phosphorus showed increases in tissue lead while a simultaneous decrease of both calcium and phosphorus content produced an additive effect

(Barltrop and Khoo, 1976). Mahaffey et al, (1973) have shown the blood lead level of rats to increase four fold, kidney lead content to increase twenty three fold and associated toxicity to increase, when dietary calcium was reduced from 0.7% to 0.1%. Calcium and phosphorus supplements reduced the gastrointestinal absorption of lead in humans, where the effect of calcium was greater than phosphorus and their combined effect was more than additive (Blake and Mann, 1983). In lambs, a reduction of blood and bone lead concentrations occurred in response to dietary phosphate supplementation (Morrison et al, 1974). Further investigations showed that phosphate and calcium supplements fed to lambs on a diet low in macro nutrients and high in lead reduced the toxicity and tissue content of lead (Morrison et al, 1977). In addition to lambs, the inverse relationship between dietary calcium and phosphorus concentration and lead uptake has also been noted in the horse (Willoughby et al, 1972) and domestic fowl (Berg et al, 1980). In general terms these interactions between calcium, phosphorus and lead take place in the gut during absorption (Quarterman, 1986) although there may be large differences between species.

As a direct result of distinctly low levels of dietary calcium and phosphorus in soils and herbage in Wanlockhead, a controlled experiment of dietary phosphorus and calcium supplementation was set up for young lambs grazing contaminated pastures the following year.

Effects of Dietary Supplements on Locomotor Disorder

The experiment showed that Vitamin C supplements did not prevent the locomotor disorder from developing at Wanlockhead and supports the findings of Aitken (1985), although the period of supplementation was short. Others have reported immediate benefits from supplementation

with Vitamin C (Clegg and Rylands, 1966) but these responses in uncontrolled trials were probably attributable to the concurrent removal of lambs from their lead-contaminated environment.

Table 31 shows only three significant negative correlations for lead and ascorbic acid over all treatments and ages. As reported earlier some clinical studies suggest a possible interaction between lead and ascorbic acid. However, like other experimental studies the results presented here would suggest that any beneficial interaction between lead and ascorbic acid is extremely tenuous indeed.

By contrast, the calcium/phosphorus supplement was highly effective. From Figure 18 it is evident that blood lead increases are less marked after one and two weeks in Ca/P supplemented lambs. Plasma calcium and phosphorus were not measured since such analyses gave normal results in Experiment 1 and may give a poor indication of body status. After treatment the lambs in this group showed no clinical signs of the locomotor disorder, although one lamb died as a result of a disorder totally unconnected with lead. All seven remaining lambs exhibited normal locomotion, good mobility, were capable of play and were easily herded without locomotor difficulty for one mile. Such contrasts with those in the control group and those receiving ascorbic acid supplements must indicate beneficial changes in the metabolism of lead by the lambs, brought about by calcium/phosphorus dosage.

In addition to earlier discussion on the benefits of calcium and phosphorus dietary supplementation, Barltrop and Khoo (1976) reported that while these nutrients decreased body lead in rats, blood lead was not affected. Blood lead levels found in lambs, although lowered, were still abnormally high and probably reflect the continued ingestion of lead. Clearly the knowledge of benefits from

calcium and phosphorus supplements may be of value in the management of the locomotor disorder on an individual and a flock basis.

Research to date on the locomotor disorder in young lambs grazing former lead mining areas has not established lead as the sole cause. Doubt has arisen largely because of the poor correlations which have been found between the lead status of the lambs and the incidence of the disorder. Even in the present work, no significant correlations were determined between blood lead and visible signs of the locomotor disorder and/or death at any age in Wanlockhead. Blood lead has though been shown to have a better predictive than diagnostic role in the present former lead mining area study. The success of treatment with major nutrients helps to explain the anomalies associated with the disorder, highlighting that factors other than lead in both soils, plants and animals will influence the quantity of lead available for absorption in the gastrointestinal tract of the lambs.

4. RESTORATION OF METALLIFEROUS MINE WASTE

4.1 DETAILED OBJECTIVES

In view of the environmental evidence, lamb disorders and deaths and elevated blood lead levels found in the inhabitants of Leadhills and Wanlockhead, an approach was made to the Scottish Development Agency's (SDA) Land Renewal team in 1984 for consideration of funding for restoration of the lead waste bings which disperse contaminated dust.

In formulating proposals for carrying out restoration of mine waste the aim was to :-

1. Eradicate water and airborne lead contamination by creating a permanent low maintenance and cost effective grass sward.

2. Remove industrial scars and produce an aesthetically acceptable environment through regrading and landscaping, consistent with surrounding land.

Proposals for future maintenance and site operations are included at the end of this section.

4.2 METHODOLOGY

4.2.1 Initial Proposal

Agreement was reached with the SDA to undertake experimental trial work in this field, and a review of existing sites of metalliferous waste totalling some 500,000 tonnes was made in Leadhills and Wanlockhead. From this it was established that the majority of tips were comprised of coarse material discarded during the preliminary stages of ore processing which was inevitably more stable than the finely ground material which had

been further processed.

One particular site close to a Primary School was earmarked for trial operations in Leadhills (Figure 19). This particular tip was made up largely of fine sand and silt which underwent erosion by wind and water especially in the summer months when the material dried out. Formerly a tailing ponds plant for the lead industry, a survey of the 60,000 tonnes of material was carried out and representative samples collected for analysis to determine the physical and chemical nature of the waste (Moffat, 1984; Richards, Moorehead and Laing Ltd, 1985).

Results for heavy metals and macronutrients in the spoil material are shown in Table 32.

4.2.2 Consultations

A meeting was set up with the landowners, Hopetoun Estates to gain their permission for land restoration. Further local consultation was carried out through meetings with Clydesdale District Council's Planning and Environmental Health departments, and Leadhills Community Council. The motion met with approval from these bodies and was further backed by the local Doctors.

Meetings with consultants acting for the Welsh Development Agency were organised to investigate methods of reclamation of this type which had been set up in N. Wales from 1975. Lead and zinc values in the waste there were found to be far in excess of those which commercial species of plants are capable of growing on. Extended trials involving experimental plots with cover materials over the spoil at between 150 and 900mm were therefore implemented in Wales to determine the best, most cost-effective barrier between the toxic spoil and planned vegetation.

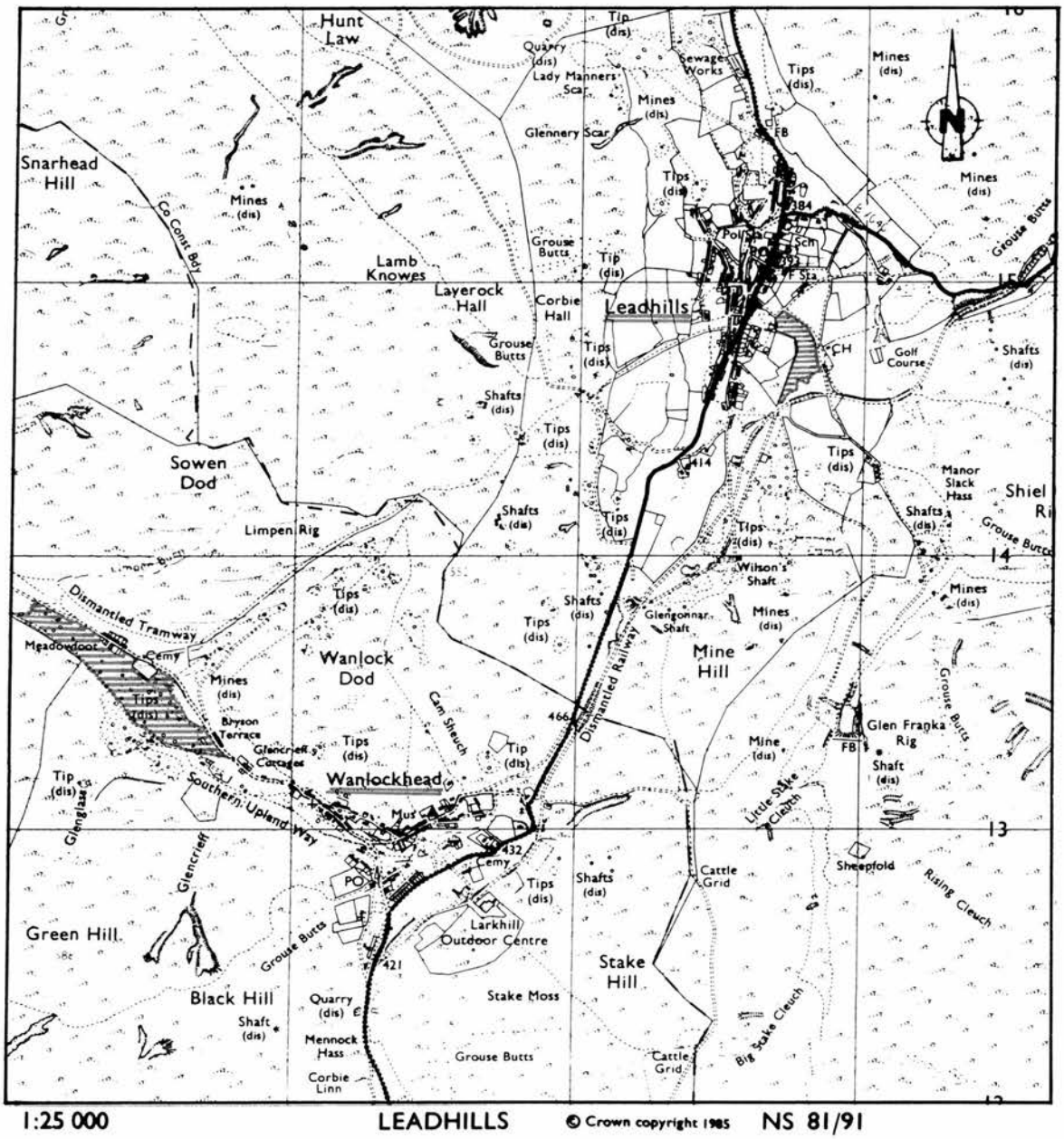


Figure 19. Location Plan: Spoil Restoration.

TABLE 32

Typical Total Heavy Metal and Available Nutrient Analysis of Spoil ($mgkg^{-1}$).

		Normal ranges in soil
Lead	10,000 - 20,000 (VH)	2 - 200
Zinc	20,000 - 50,000 (VH)	10 - 300
Copper	130 - 530 (M-H)	25 - 200
Cadmium	99 - 209 (VH)	1 - 4
Nitrogen	2 - 3 (VL)	200 - 2,000
Phosphorous	26 - 37 (M)	10 - 50
Potassium	26 - 45 (VL)	40 - 1,000
Calcium	9,000 - 13,000 (H)	1,000 - 3,000
Magnesium	120 - 200 (M)	20 - 1,000

Note 1: VL - Very Low; M - Medium; H - High; VH - Very High.

2: pH : 8.1

Amendments on trial included steel slag, unburnt coal shale, domestic refuse, top soil, and burnt colliery shale, either with or without sewage sludge additions (Robinson, Jones Partnership, 1977).

The Welsh results from two years of research trials using the above cover materials can be summarised as follows :-

After 1 year:-

1. Control plots seeded directly onto metalliferous waste failed and did not respond to fertilisation.

2. Grass cover on blast-furnace slag did not progress beyond germination since the material was too free-draining.

3. Evidence was found of lead and zinc affecting grasses on plots at 150mm and 300mm thickness of topsoil, largely because of rapid root penetration into toxic waste.

4. The performance of plots using coal shale were most encouraging with the material permitting only shallow root growth.

After 2 years:-

1. The highly fertile amendments of topsoil, domestic refuse and colliery spoil with sewage sludge were most productive.

2. Deterioration occurred in the swards at 150mm and 300mm thickness of topsoil and domestic refuse with high metal contents evident. Root penetration had become extensive and had reached toxic spoil in the 450mm plots of fertile amendments.

3. Plots established on infertile amendments (both types of colliery spoil) showed no progressive deterioration and increased levels of metal contamination were found on only the 150mm beds.

A further site visit was made to the Conway Valley of Wales where the Park lead mine waste was reclaimed in 1978, using a 375mm cover of subsoil material brought from an adjacent borrow tip. Grass establishment was reasonably successful, although a long term commitment of maintenance / fertilisation lasting a minimum of five years was deemed necessary. Animal grazing was suspended after two years because of damage to the sward and toxicity of vegetation. Recommendations were made to fence sites for reclamation in order to eliminate grazing damage.

An overview and examination of the results, successes and failures of these trials, and liaison with the University of Liverpool who were engaged in research in this field, were applied to the Leadhills site (M.S. Johnson and P.D. Putwain, 1984/5, Personal Communication).

4.2.3 Existing Site Conditions

Early investigation included the assessment of alternative potential solutions including removal of waste material to alternative sites, but it was felt that as well as 'transporting' the problem, this would lead to further erosion and dispersal of toxic material locally. Reprocessing of mine waste was investigated by Richards, Moorehead and Laing (1985)to examine the commercial viability of extracting valuable minerals from the wastes. This too was ruled out on the basis of further environmental contamination.

Natural revegetation of the 2 hectare site in Leadhills has never occurred since the closure of the mines in the

1930s. This is usually the case in such spoils because high levels of zinc and lead are phytotoxic to plants. Of these, zinc is most phytotoxic since compounds of zinc pass more readily into the soil solution from which the ions are extracted by the growing plant. Lead too may be toxic for growth, but usually only when calcium levels are low. Of equal importance is the low fertility of the spoil in terms of macronutrients which are essential for plant growth. The organic matter content of the waste is negligible since there is no properly developed soil for long term storage of nutrients or a labile pool related to the ion-exchange capacity of the material. These are both essential for plant growth.

4.2.4 Reclamation Proposals

Landscaping

Earth moving works began in 1984 (Figures 20 and 21). These included demolition and site clearance; remodelling of the spoil heaps to a stable landform; and the installation of a temporary open ditch drainage system with silt traps to intercept large volumes of contaminated silt from site run off in the early stages. The site was then completely fenced-off and made rabbit-proof to avoid serious sward deterioration on seeding.

On site completion trial plots were constructed in June 1985 over a small area using cover materials close to hand. Despite survival being thought unlikely, costs were justified to attempt full scale direct hydroseeding trials of the metalliferous waste, and these were also implemented in 1985 using various mixes of grasses and mulches.

Figure 20. Spoil Before Restoration.

Figure 21. Spoil During Restoration.

Trial Plots

A number of proposed cover materials were investigated locally. For example, local peat was examined and found inadequate since on removal from a wet bog situation, oxidation and moisture losses would have led to an 80 per cent decomposition of the material. The use of a plastic sheeting cover was also ruled to be unsatisfactory because of obvious problems of puncturing and 'slip'.

Since a low pressure economic amenity land use was intended, a highly productive vegetation cover was of secondary importance and less fertile amendments more conducive to the development of a minimal maintenance system requiring little attention in the long term were highlighted. These finally included (1) Local subsoil (pH 5.5), (2) Well degraded local domestic refuse some 25 years old (pH 5.8) and (3) Colliery spoil from a nearby tip (pH 5.0).

An area typical in terms of heavy metal and macronutrient content of the whole site was earmarked and chosen for trial plot establishment. Experimental plots at depths of amendments ranging from 150mm to 450mm were implemented using the above three materials.

Prior to introducing the amendments, each plot was dug 2 metres by 1 metre and lined with a 500 gauge polythene sheet to prevent lateral movement of materials with the bottom left open to allow drainage of the spoil. Overall, an extra 20% of each cover material was added to every plot depth to account for compaction of materials.

36 experimental plots (Figure 22) were constructed at three depths for local soil and domestic refuse and at two depths for colliery spoil, the depth of the latter being curtailed by haulage costs. A duplicate treatment/depth combination was established for every situation. No preparation was required for the four

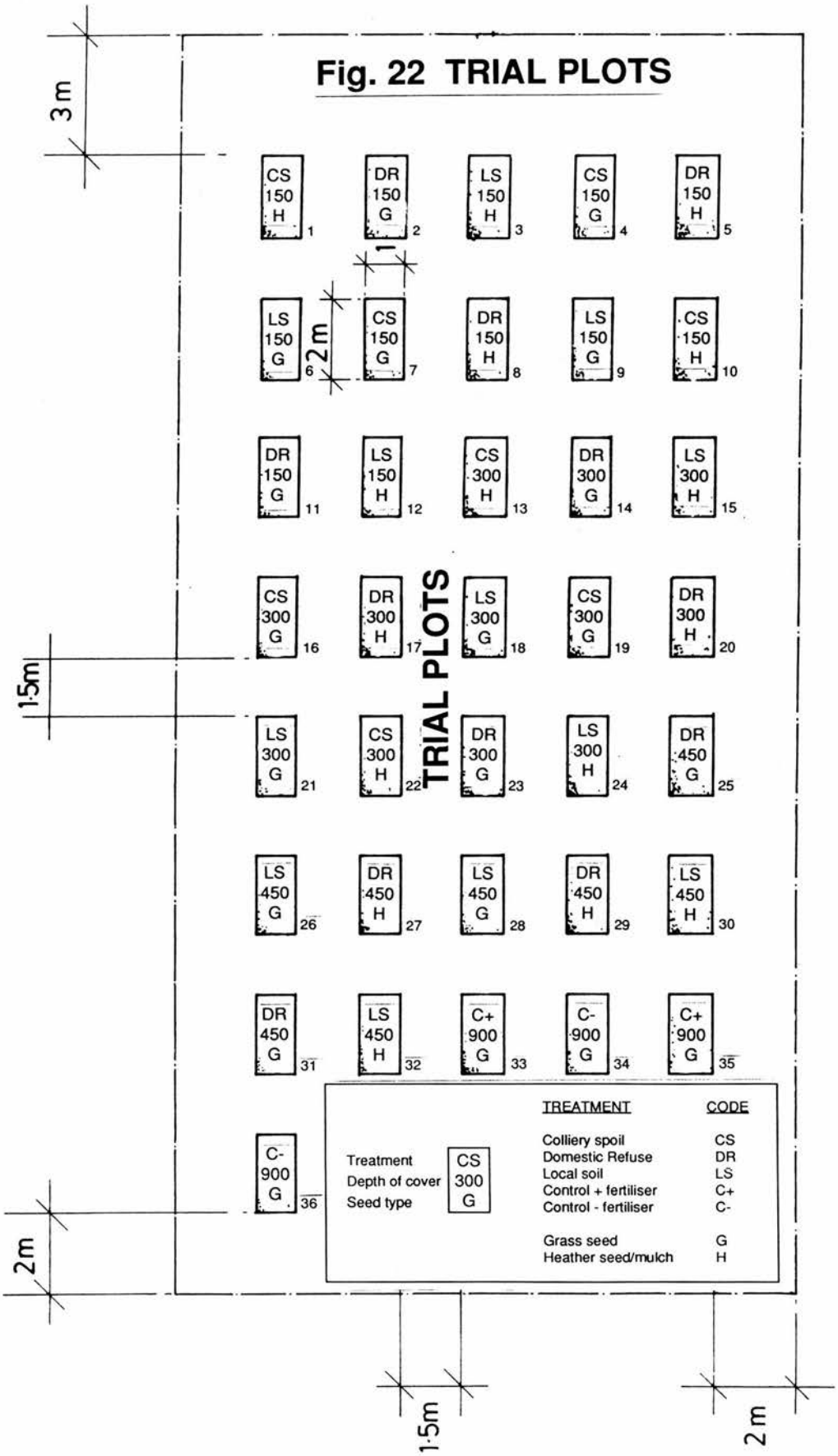
control plots which were introduced.

Each finished plot was lightly raked and stone picked with half the plots receiving a grass seed mix given a basic application of fertiliser (N, P_2O_5 and K_2O at 50 $kgha^{-1}$) incorporated into the top 1-2" of each plot. This fertiliser application was repeated on one occasion the following year at the beginning of the growing season. Colliery spoil plots were limed in the top 6" at 7,500 $kgha^{-1}$ to overcome existing and potential acidity. The remaining plots due to be seeded with locally collected heather seed and litter were given no fertiliser and no lime additions.

Grass seed mixtures consisted of 30% Festuca rubra Merlin (tolerant to high levels of lead and zinc); 15% Highland brown top; 15% Poa compressa Reubens; 10% Festuca ovina; 15% Festuca longifolia Scaldis; 10% Trifolium repens; and 5% Lotus corniculatus at 65 $kgha^{-1}$. Overall, the grass mix was designed to provide facility for high yielding tolerant species; less productive and resilient grasses; and grasses capable of counteracting arid ground situations and nitrogen deficiency. For plots receiving a heather mix, one square metre of each plot was seeded with (a) Heather Seed and (b) Heather litter, with 25 $kgha^{-1}$ of Highland brown top. Seedling establishment was recorded using a 500 x 500mm quadrat which was subdivided into units of 100sq cm and positioned twice on every plot.

Each plot was harvested in early September by cutting a constant proportion of the surface growth of each plot, some 5cm above ground level to avoid contamination by soil splash. This was used to give an overall indication of productivity and then transferred to the laboratory where samples were washed to remove surface contamination, prior to analysis for heavy metals and macronutrients.

Fig. 22 TRIAL PLOTS



*Hydroseeding Trials

Having completed the plots, immediate full scale restoration of the two hectares of spoil using direct hydroseeding was attempted. An investigation of research methods elsewhere indicated that such a method would fail even with sufficient fertiliser applications unless specially bred 'tolerant' grasses were used to withstand toxic heavy metals. Nevertheless, direct hydroseeding was seen as a short term means of minimizing dust nuisance in terms of health, while trials to determine the most effective long term cover material for the site were investigated in the following years from the trial plot investigations. The full scale proposals for hydroseeding also involved controlled trials, including three different seed trials and two mulches (Figures 23 and 24).

The overall area was split into two sites A and B largely as a result of ground conditions since Site A had six times more silt and half the amount of coarse sand compared to Site B, and clearly had a higher moisture potential. Each seed mulch mix used in Site A was replicated in Site B, with each grass seed mix replicated within each of sites A & B using the alternative mulch.

Included in 50% of the grass mixes in Trials 1 and 2 were the seeds of Festuca rubra Merlin known to be tolerant to high levels of lead and zinc, and bred from similarly toxic land in Wales; 25% of Poa compressa Reubens was also added for its adaptability to excessive levels of lead and zinc. Trial 3 was composed of a Commercial (reclamation) grass seed mix and acted as a 'Control' for the project.

* Hydroseeding is a technique for the rapid site application of seeds, fertiliser and mulches using specialised hydraulic seeding machinery consisting of a tank fitted with a slurry pump, agitator paddles, hoses and a demountable jet. This machinery is usually mounted on a lorry.

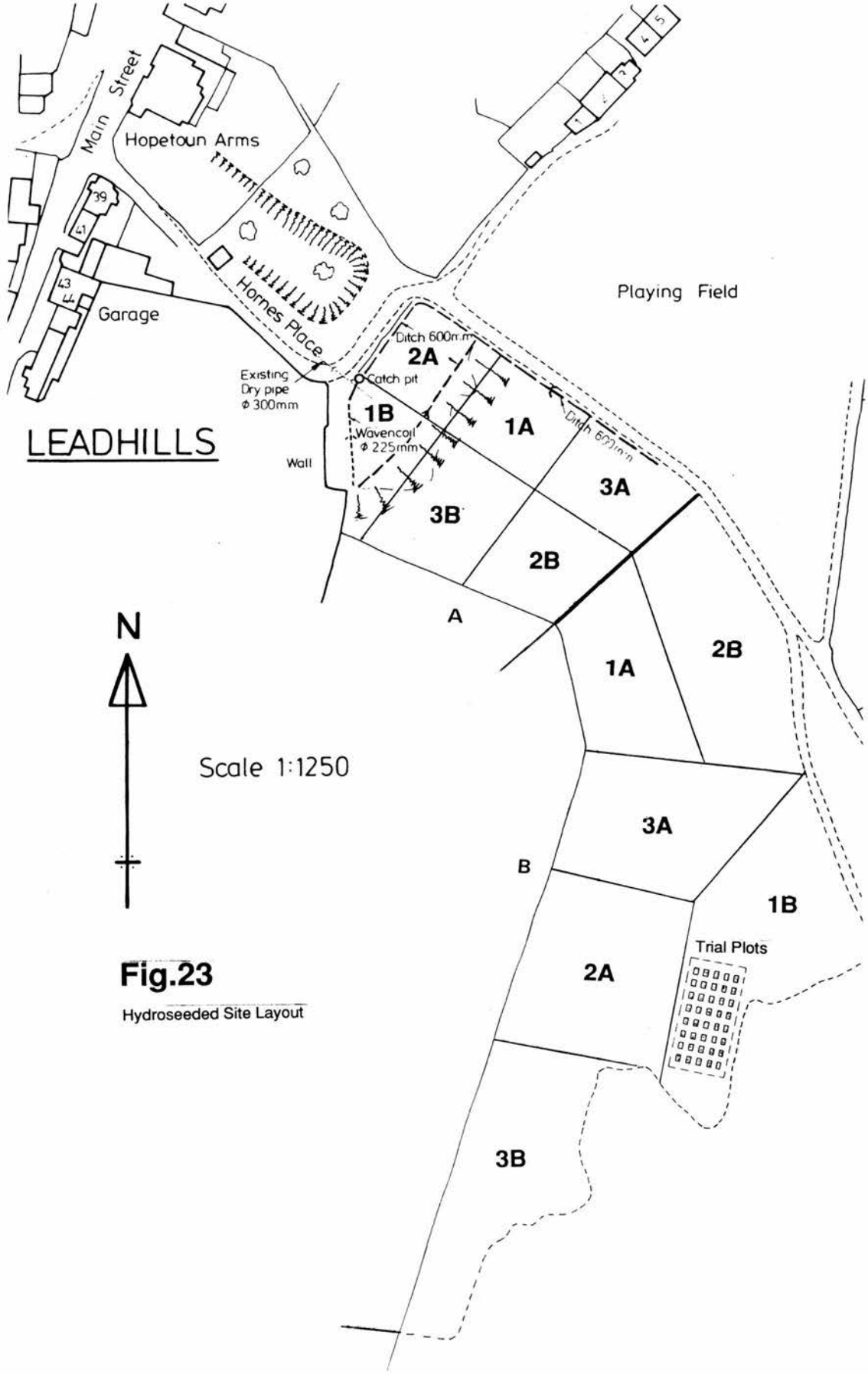


Fig.23

Hydroseeded Site Layout

TRIAL 1A) Grass Seed @ 150 Kg/ha

50%	Festuca rubra Merlin - Lead/Zinc Tolerant
10%	Agrostics tenuis Highland
25%	Poa compressa Reubens
10%	Festuca longifolia Scaldis
5%	Festuca ovina

Wood Cellulose Mulch	@ 1500	Kg/ha
17:17:17 Fertiliser	@ 300	Kg/ha
Enmag Slow Release	@ 150	Kg/ha
Soil Stabiliser Huls 801	@ 200	Kg/ha

TRIAL 1B) As above except with 3000 Kg/ha Peat Mulch instead of Wood Cellulose Mulch.

TRIAL 2A) Grass Seed @ 150 Kg/ha

50%	Festuca rubra Merlin - Lead/Zinc Tolerant
25%	Poa compressa Reubens
10%	Agrostis tenuis Parrys - Copper Tolerant
10%	Poa pratensis Parade
5%	Westerwolds rye grass - Nurse Crop

Wood Cellulose Mulch	@ 1500	Kg/ha
17:17:17 Fertiliser	@ 300	Kg/ha
Enmag Slow Release	@ 150	Kg/ha
Soil Stabiliser Huls 801	@ 200	Kg/ha

TRIAL 2B) As above except with 3000 Kg/ha Peat Mulch instead of Wood Cellulose Mulch

TRIAL 3A) 'Reclamation' Grass Seed @ 150 Kg/ha

20%	Hard fescue Tournament
20%	Sheeps fescue
35%	Creeping red fescue Boreal
15%	Poa compressa Reubens
7.5%	Browntop bent Highland
2.5%	White clover

Wood Cellulose Mulch	@ 1500	Kg/ha
17:17:17 Fertiliser	@ 300	Kg/ha
Enmag Slow Release	@ 150	Kg/ha
Huls 801 Stabiliser	@ 200	Kg/ha

TRIAL 3B) As above except with 3000 Kg/ha Peat Mulch instead of Wood Cellulose Mulch

Figure 24. Trial Hydroseeding Proposals

Each mix was sown with both a Wood cellulose mulch at a rate of 1500 $kgha^{-1}$ and Peat mulch at a rate of 3000 $kgha^{-1}$. Grass seeds were sown at 150 $kgha^{-1}$, together with 300 $kgha^{-1}$ 17:17:17 fertiliser, 150 $kgha^{-1}$ Enmag slow release, and 200 $kgha^{-1}$ soils stabiliser Huls 801. The last of these was particularly important in what is a very exposed site at 1500 feet susceptible to high winds. All trial plots were covered with polythene during hydroseeding operations. By late 1985 it became clear from initial herbage growth that the Contractor had made a number of errors in both seeding and mulch mixes, and the decision was taken to weedkill and re-hydroseed in the spring of 1986 in accordance with original specifications.

Herbage sampling was employed using a standard 'W' technique with five bulk samples collected from each trial area. Other analysis and sampling methods were as outlined earlier for trial plots. Laboratory and investigational techniques throughout this work are as reported in the 'Lamb Study' with similar precautions implemented to avoid error and bias.

From 1985 to 1990 inclusive each trial plot and hydroseeded area was monitored on four counts. The first three of these - percentage cover, yield and sward composition - were designed to provide an assessment of the productivity and visual acceptance of the sward, together with an indication of maintenance requirements. The fourth factor involved monitoring of heavy metals and macronutrients in the grasses to determine the agricultural value of the sward and the likelihood of sward deterioration. Root cores were also taken to determine the maximum rooting depth.

4.3 RESULTS

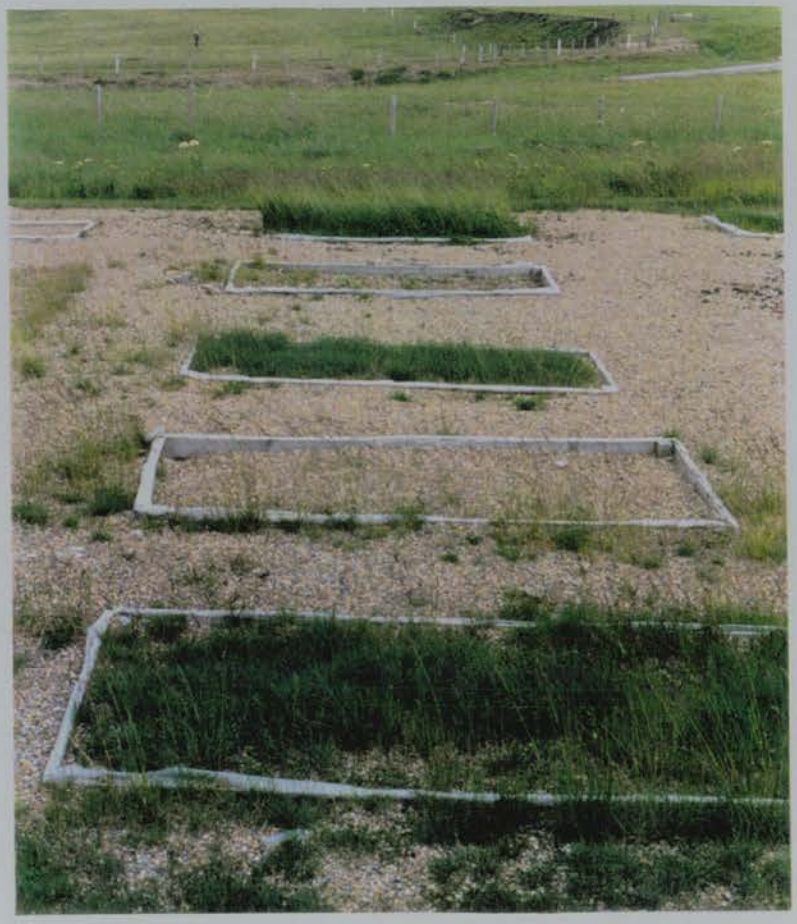
4.3.1 Trial Plots

Successful germination took place on all plots seeded with the grass seed mix, with no distinction evident between amendment types. Those plots seeded with heather litter and heather seed failed to germinate regardless of amendment. An acceptable ground cover and grass sward composition of more than 85 per cent took place on all three amendments regardless of depth to which they were applied (Figure 25).

In yield terms, the herbage showed most initial promise on the colliery spoil amendment with no significant differences evident between domestic refuse and local soil. Control plots seeded directly on to metalliferous spoil, showed clear differences in yield and ground cover according to fertiliser application. From 1986 to 1989 it became clear that plots amended with domestic refuse and colliery spoil were most successful in terms of ground cover and yield compared to local soil.

The results of heavy metal analysis are shown in Figure 26 and Appendix 3. There were no overall significant differences for pooled heavy metals in herbage according to depth of amendment cover although an examination of root cores showed metal levels were often closely related to root penetration. Only cadmium was found to be significantly lower ($p<0.01$) in herbage at 300 mm compared with the 150 mm depths. On a general basis, there was a tendency over the four years 1986-1989 for local soil herbage to accumulate more lead ($p<0.05$) and cadmium ($p<0.05$) probably due to the ease of swift root growth and penetration in the soil matrix, and because the soil being local, already contained heavy metal contamination.

Figure 25. Trial Plot Herbage.



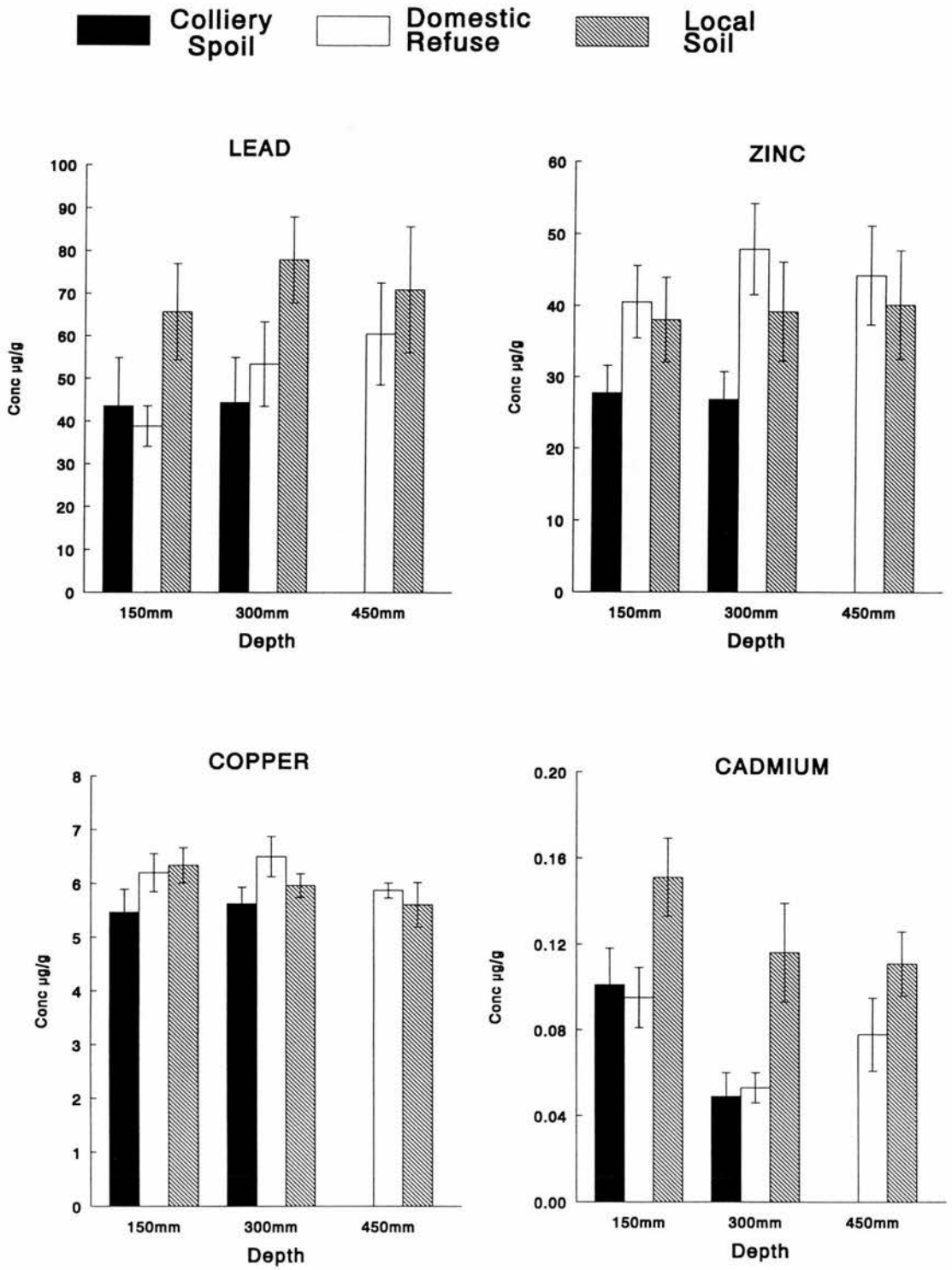


Figure 26. Accumulation of heavy metals in trial plot herbage (Means ±SE for pooled data 1986-1989, n=8)

Moreover, the structure of soil would allow the vertical movement of heavy metal salts by capillary action. Zinc concentrations were significantly lower in colliery spoil herbage ($p<0.05$).

It was evident that cover materials at depths of 150, 300 and 450mm do little to minimize metal uptakes into vegetation. Lead concentrations in herbage were very high throughout, although zinc, copper and cadmium concentrations remained within normal limits. Harvest collections were not implemented in 1990.

The control plots directly seeded onto spoil material accumulated between two and three times the levels of lead, zinc, and in some instances cadmium, compared to those seeded onto amendment covers. Interestingly, all seed types, including legumes survived for three years on directly seeded plots where a fertiliser addition was given for the first two years. Plots seeded directly onto spoil with no fertiliser addition failed to survive in the second year, with phytotoxicity and nutrient deficiency evident.

Despite uptake of heavy metals into vegetation, an effectively stable ground cover was achieved over the study period on all amendment plots, with no evidence of severe deterioration or phytotoxicity. Although successful in terms of stabilising the toxic spoil, it is not unexpectedly evident that much deeper depths of amendment or a much coarser amendment material to prevent penetration by roots and capillary action, would be necessary to achieve non-toxic herbage should grazing have been envisaged. In this particular case, such an exercise was financially limiting in terms of remedial costs.

4.3.2 Hydroseeding

First hydroseeded in July 1985, the whole site initially germinated satisfactorily regardless of seed mix or mulch applied. However, within eight weeks it was evident growth was not progressing in an acceptable fashion especially on the sloped, more exposed site B (20-30% cover). This was explained by excessively heavy rainfall over this period which was responsible for both leaching of fertiliser and washing away of seed. Site A's growth was markedly better (80-90% cover) due to its more suitable growth matrix with a high percentage of silt retaining both fertiliser and seeds more easily. This situation, coupled with the contractual errors outlined earlier led to the site being weedkilled and re-seeded in 1986.

By the end of the first growing season (1986) the following was established:

1. Seed mix A's performance (in terms of ground cover and growth) was marginally better than B, with commercial mix C giving the poorest results.

2. Wood mulch appeared best suited to the area for establishment and initial growth, when compared to peat.

3. Latterly, that the site was as a whole, deficient in fertiliser. This was confirmed by 'spot fertilisation' trials.

4. Having been seeded late in the season, herbage growth was not sufficient for adequate or accurate measurement of heavy metals and macronutrients.

A further application of artificial fertiliser in 1987 ensured another years satisfactory growth and ground cover with no signs of phytotoxicity. The proposal in 1988, in view of the previous years 'success' was

therefore to enhance the main site growth through sewage sludge applications as 'top' dressings to bring levels of organic materials/nutrients up to recommended levels in the spoil for long term growth.

Consultation and liaison with the Water Research Centre (WRC) led to three separate applications at the beginning of growing seasons 1988, 1989 and 1990. Containing useful quantities of nitrogen, phosphorus and organic matter sewage sludge is also a well balanced manure for restoring disturbed soils. Furthermore, it afforded the potential for maintaining the high site pH since sludge is usually above pH 6.5, and has a high pH buffer capacity to maintain its overall high level. Nutrients from sewage sludge are released over several years, and give better soil structure, porosity, aeration, drainage and water storage capacity affecting the rate of organic decomposition and the cycling of materials.

Over 3 years, the aim was to achieve an addition of over 150t dry solids/ha to achieve substantial long term effects for herbage growth. The average composition of the sewage sludge added is shown in Table 33. Fears of adding further heavy metals from sewage sludge to the final soil were negligible, since being many times lower in the sludge they in effect diluted the concentration of metals in the top soil of the spoil. Application of cake sludge was made by conventional muckspreaders using sludge from Dumfries and Galloway in 1988; Shotts, Strathclyde in 1989; and Daldowie, Strathclyde in 1990. Good ground conditions were always required to avoid excessive wheeling damage to the Sward on site.

Ground conditions and cover/yield remained highly successful over all years using this method, with no phytotoxicity evident.

TABLE 33

Typical Sewage Sludge Analysis for Heavy Metals ($mgkg^{-1}$) and Macronutrients (%) 1988-1990.

Lead	570	(250 - 902)
Zinc	677	(411 - 988)
Copper	332	(165 - 461)
Cadmium	4.0	(3.8 - 4.2)
Chromium	133	(89 - 181)
Nickel	51	(36 - 79)
Manganese	604	(561 - 749)
Mercury	2.0	(0.8 - 3.3)
Nitrogen	1.20	(0.34 - 1.86)
Phosphorus	0.85	(0.22 - 1.93)
Potassium	0.12	(0.07 - 0.16)
pH	7.4	(6.5 - 8.2)

Developing Sward required little maintenance in terms of cutting in the first two years and was curtailed through sheep grazing in the final years (see following "Sheep Grazing Trials").

A breakdown of heavy metals and macronutrients in herbage for the years 1987-1990 are shown in Figures 27 and 28, and Appendix 4. Results are presented according to overall mean values for each trial seed mix, since no appreciable difference was found in parameters between sites A and B or between mulch types originally applied.

No significant differences were determined for heavy metals in herbage from Trials 1 and 2 over these four years. Furthermore, no conclusive evidence was found for commercial seed mix 3 accumulating higher concentrations of heavy metals compared to tolerant mixes 1 and 2. Nevertheless, examination of results show lead and zinc to be highest in 1987 and 1989 (both $p<0.01$), as well as cadmium in 1989 ($p<0.05$) in mix 3. These results bear some relationship with the corresponding metals in sludge applied for 1988-1990. Although some herbage zinc concentrations exceeded the normal upper limit and lead concentrations were relatively high, all seed types, including clover survived the trials (Figure 29). Copper and cadmium concentrations remained within normal limits throughout.

Macronutrient levels remained largely stable over the first three years in all trials although in 1990, nitrogen levels were lower ($p<0.001$) and would appear to be related to a quarter of the nutrient present in that particular years sludge application, compared to applications in the previous two years. Potassium levels were similarly depleted.

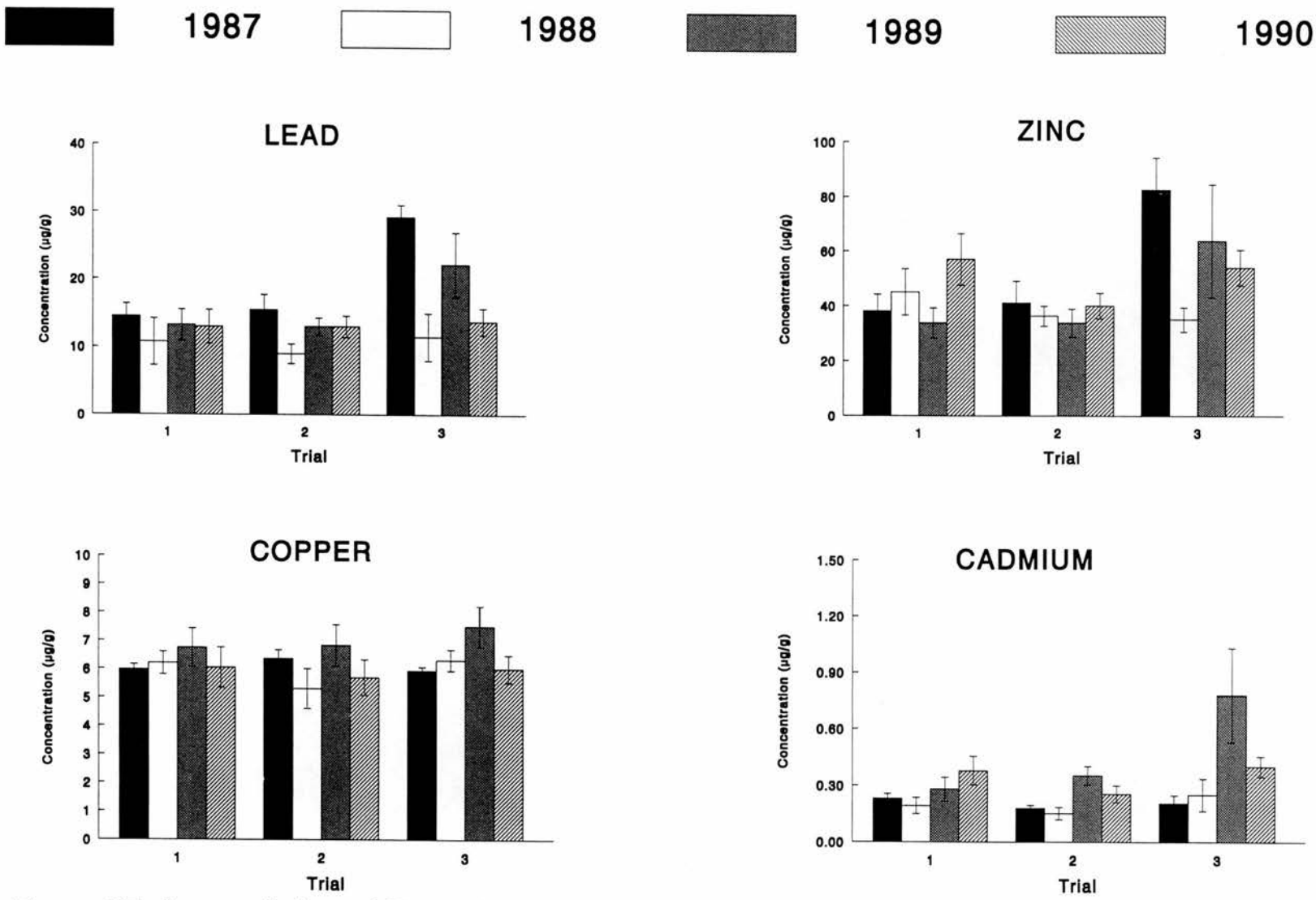


Figure 27. Accumulation of Heavy Metals in herbage (Means ±SE, n=4): hydoseeding trials.

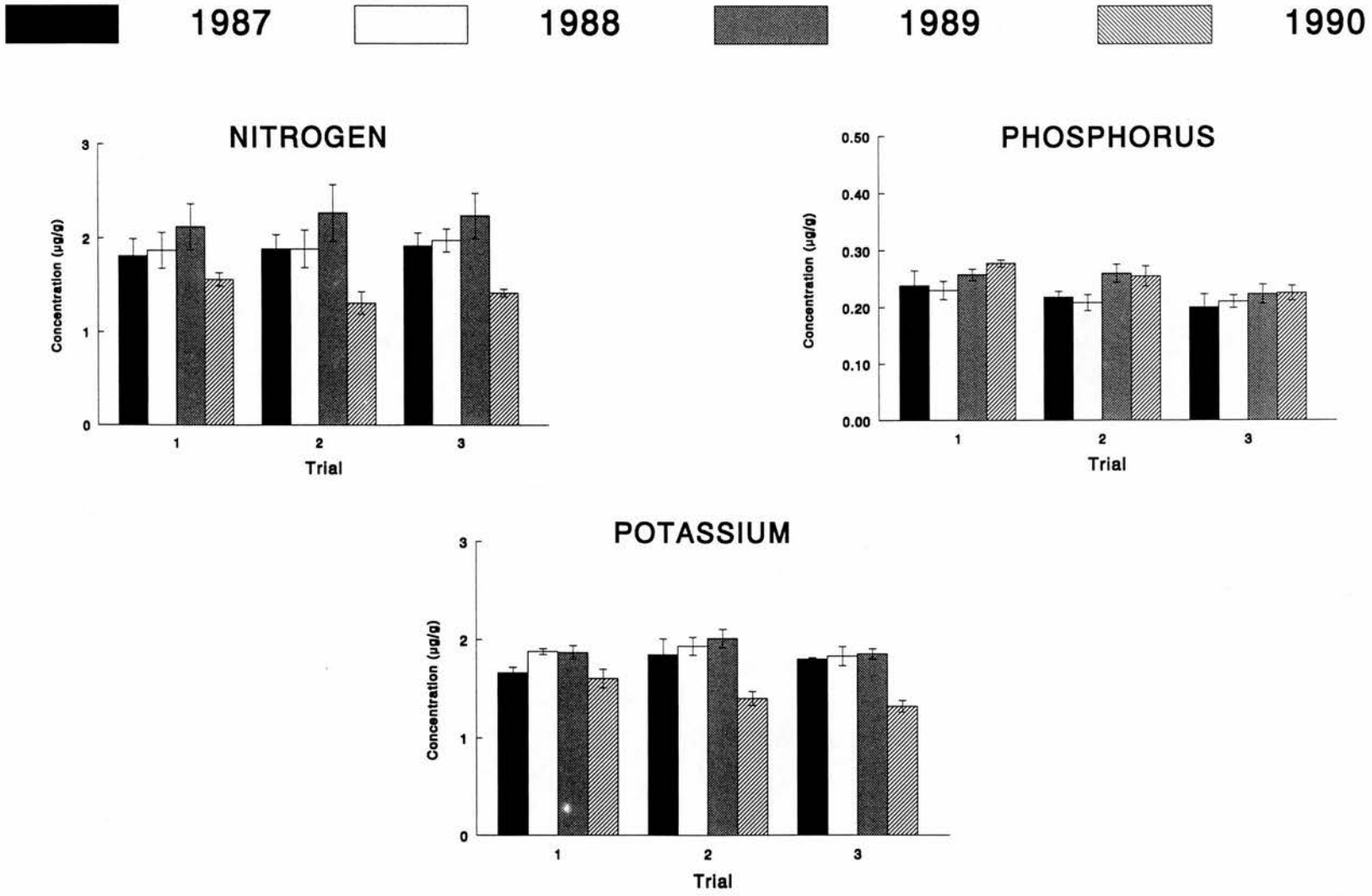


Figure 28. Accumulation of Macronutrients in herbage (Means ±SE, n=4): hydoseeding trials.

Figure 29. Hydroseeded Herbage.

Prior to this work, and according to work in trials carried out in North Wales it was very unlikely that commercial grasses would survive beyond 2 years on directly seeded spoil of this kind and only tolerant plant species would succeed because they are able to render the toxic metals metabolically inactive by complexing them in the root cell walls (Borough of Wrexham Maelor and Montgomery District Council). In the present work, all grass mixes including the standard commercial mix have survived and shown productivity over a seven year period. It is believed that this situation has occurred largely as a result of the high site pH and the eventual formation of an organic matrix.

Firstly, it is likely that when fertiliser and sewage sludge were added that phosphorus played an important part in regulating the uptake of heavy metals in plants by forming insoluble heavy metal-phosphate complexes. Secondly, and perhaps more importantly the pH of the spoil was very high with high levels of calcium influencing the availability of heavy metals to plants. Heavy metal accumulation was lower overall in herbage from the hydroseeding trials compared to those in the trial plot work. It is interesting to note that Welsh trials using direct seeding failed on an acidic spoil with a pH of 4.2-4.7; and on calcareous spoil with a pH of around 7. While the latter situation may be explained by differences in spoil zinc availability, the higher pH on the Leadhills site is clearly very important for long term herbage survival.

4.4 SHEEP GRAZING TRIALS

In view of the unexpected relatively low metal levels in herbage, and in order to help maintain a grass cover, controlled sheep grazing trials were put into operation from late July to early October (1989) on the restored site, after consultation with Hopetoun Estate's Farm Manager.

Two groups of ten Blackface sheep aged two years old were randomly selected with one group placed on the restored site and the other on land seven miles outwith the geological lead area. Despite this, the control area had over many years been mildly contaminated by lead through wind and water dispersal from former industry. It nevertheless acted as an effective 'control' area in view of the high metal levels on the restored site. Typical geometric means and 95% ranges of lead, zinc and copper in soils and unwashed herbage on the two sites are shown in Table 34.

Blood lead, zinc and copper were measured prior to sheep being placed on-site, after 1 week and then every second week thereafter for 11 weeks in all. This covered the calendar dates 20 July 1989 to 6 October 1989. The results of this exercise are shown in Figure 30 and Appendix 5. Blood lead levels in sheep from the Leadhills site were significantly higher throughout the specified time period rising to a concentration one and a half times greater on average compared to those from the control. Significantly lower values were observed for plasma copper in sheep from the restored site on four occasions, confirming the earlier findings for ewes in Wanlockhead. Zinc concentrations were normal on both sites.

TABLE 34

Mean Soil and Herbage Levels of Lead, Zinc and Copper on Restored And Control Land ($mgkg^{-1}$).

Soils	**pH**	**Pb**	**Zn**	**Cu**
Restored Site	8.1	18,864 (13,904 - 25,591)	8,561 (3,474 - 21,099)	180 (160 - 202)
Control	5.6	174 (154 - 197)	88 (79 - 99)	31 (26 - 36)
		***	***	***
Herbage		**Pb**	**Zn**	**Cu**
Restored Site		14.5 (10.1 - 20.8)	42.9 (36.8 - 50.2)	7.2 (6.3 - 8.2)
Control		1.4 (1.1 - 1.8)	25.4 (18.7 - 34.5)	9.2 (8.1 - 10.5)
		***	**	**

Note 1: n=6, Restored and Control land.
2: Geometric means and 95% ranges for soils and herbage obtained after natural log transformation.
3: Independent two sample T-test used to test for significance, where $^{**}p<0.01$ and $^{***}p<0.001$.

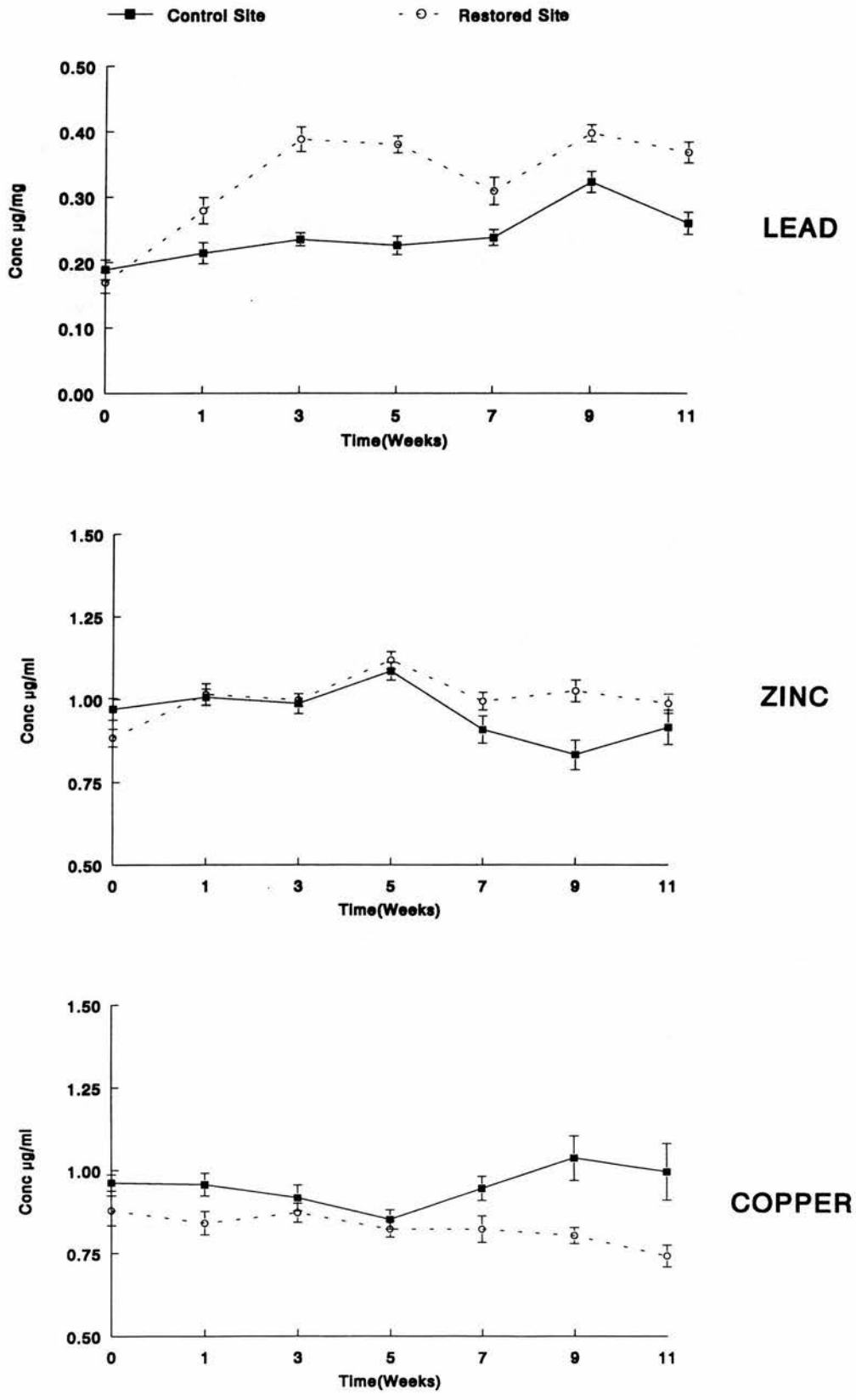


Figure 30. Grazing Sheep Blood Trials 1989 (Means ±SE, n=10)

Blood lead concentrations in sheep on the restored site were outwith the normal range of 0.05-0.25 ppm lead : no obvious ill effects were however evident in the animals. Interestingly a spot blood trial of sheep grazing neighbouring hill land with a low pH (4.8) had an essentially similar mean blood lead level of 0.40 μgg^{-1}. This occurred despite 'surrounding' acidic soils having around four times less lead.

This can probably be explained by the much greater pH of 8.1 on the restored site reducing metal uptake both into vegetation and from the animal gut.

Further, more intensive sheep trials were implemented on a similar basis the following season from 24 May 1990 to 5 October 1990. The intention was to allow measurement and investigation of the safety of sheep grazing on metalliferous sites from earlier in the year when soil ingestion is likely to be greatest, constituting up to 30% of diet when grass availability is low. Following initial blood sampling sheep were sampled two weeks after introduction to the sites and usually every three weeks thereafter. A full breakdown for each site of lead, zinc and copper in blood is shown in Figure 31 and Appendix 5 : once again blood lead concentrations in ewes on the restored site were significently higher throughout. Due to farming reluctance to place sheep on 'bare' ground near the beginning of the growing season in Leadhills it was not possible to measure any real spring soil ingestion effect. However, by the eighth week of sampling it was clear on both sites that blood leads had risen markedly. This followed a three week period of virtually no rain and little herbage growth leading to overgrazing by sheep and likely ingestion of soil. After this period there was extensive rainfall and subsequent regrowth of herbage with blood lead levels falling accordingly.

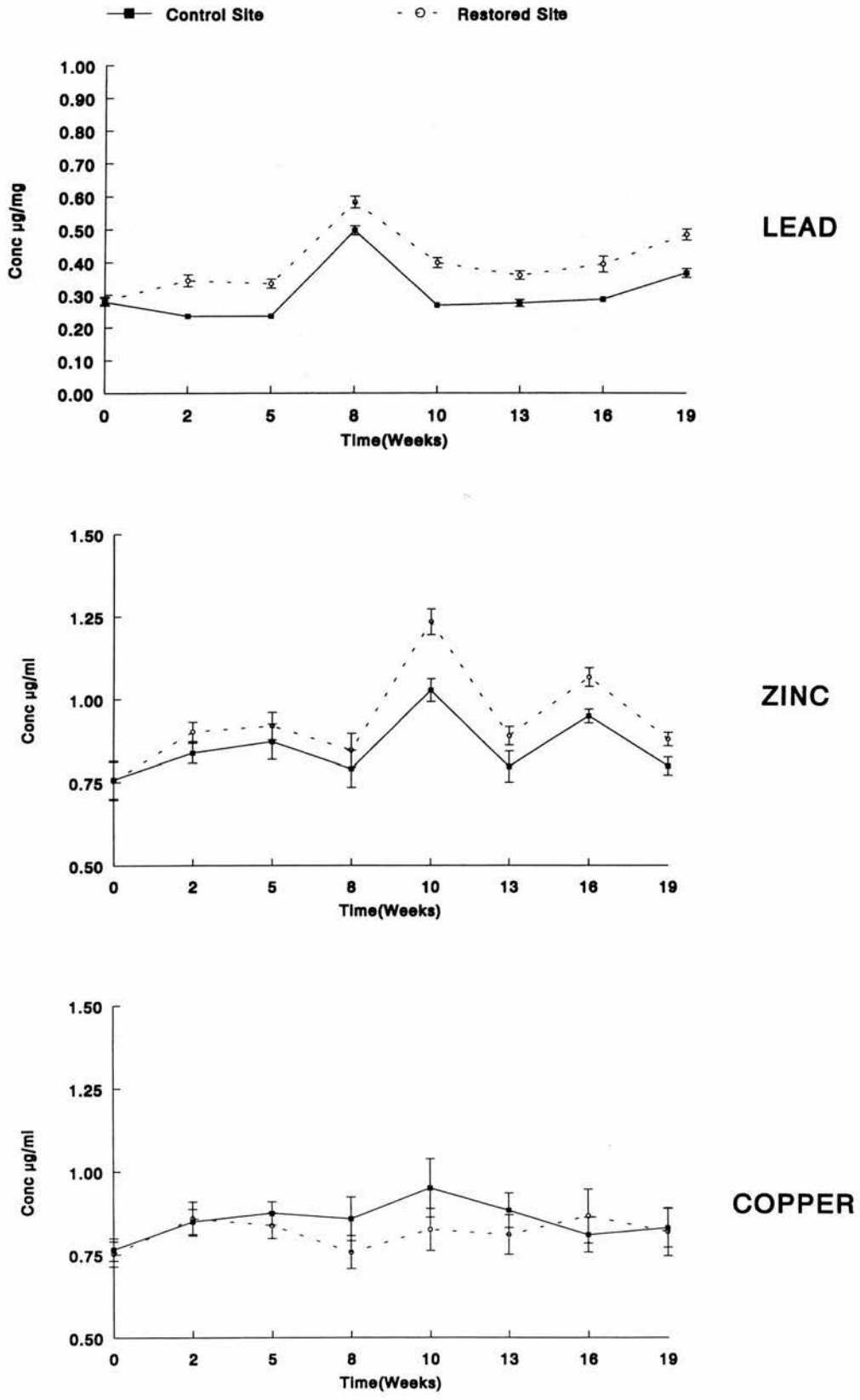


Figure 31. Grazing Sheep Blood Trials 1990 (Means ±SE, n=10)

With herbage growth again curbed by climatic conditions in the fall of the year blood leads rose again slightly. As well as soil ingestion occurring,it is likely that herbage heavy metals had increased due to translocation from the root to the shoots in the non-growth period. No significant differences were found for plasma copper between areas although it is evident some sheep were slightly deficient between 8 and 19 weeks and between 10 and 19 weeks on the restored and control site, respectively. Plasma zinc levels although significantly higher in Leadhills on three occasions remained normal throughout in both areas.

4.5 DISCUSSION

The objective of the reclamation research was the eventual creation of an ecologically stable vegetation system on metalliferous toxic waste which will perpetuate itself in the absence of ongoing nutrient amendment. Whether this is possible in the long term remains to be seen since much research has yet to be done on the decomposition of organic matter and its subsequent mineralisation and nutrient re-cycling in the presence of heavy metals. The situation in Leadhills as of Spring 1993 is however very promising.

4.5.1 Trial Plots

(a) A productive grass sward was established on all trial plots regardless of amendment type or depth, though most successful were those plots amended with domestic refuse and colliery spoil. All plots seeded with heather failed.

(b) Control plots seeded directly onto metalliferous spoil survived for three years following two additions of artificial fertiliser but showed signs of sward deterioration and phytotoxicity. Control plots seeded without fertiliser survived only into the second year before complete deterioration.

(c) Heavy metal accumulation in vegetation did not cause progressive deterioration of the sward. However concentrations of heavy metals in the trial plot vegetation were high and would preclude management by grazing.

(d) The technique of trial plot/amendment cover reclamation was successful in terms of removing the problem of toxic waste dispersal by wind and water erosion.

4.5.2 Hydroseeded Trials

(a) All hydroseeded sites, seeded directly onto metalliferous spoil succeeded over the five year study period. Success was achieved using all seed/mulch mixes, including a conventional reclamation seed mix with no 'tolerant' species added. Grass cover developed best in the first three years using seed mixes A and to a lesser extent B, and a wood mulch application. Seed mix C, had established well by the end of the fifth year.

(b) Growth was supported by artificial fertiliser application in the first two years to deter nutrient deficiency and phytotoxicity. Over the final three years trials, a total of 150 tds ha^{-1} of sewage sludge was surface spread over all areas in order to develop a long-term organic matrix complex, and to encourage growth. Soil analysis as of 1990 indicated an organic soil structure to have formed.

(c) Lead accumulation in herbage was on average 3 to 7 times lower than in the trial plots. Short periods of heavy grazing for adult sheep is possible, although metal levels in herbage should be periodically determined.

(d) An overall effective sward presently covers the former tailing ponds, eradicating dispersal of toxic material through wind and water erosion.

The Leadhills research has provided a specification for further hydroseeding on a similar 6.25 hectare site in Wanlockhead in 1990/91 (Figure 19). Here, care was taken in earth moving works to maintain/re-form the historical interest of four tailings ponds. Sewage sludge was applied at the outset in one addition of 150 tds ha^{-1} according to financial availability. The long term outcome is at present unknown, although there is some concern that since soil fertility has not been built up slowly an initial dramatic growth effect may not be sustained. Results at present (Spring, 1993) are however encouraging.

4.5.3 Future Maintenance and Site Operations

(1) Maintenance of fencing and control of vermin should be implemented. Burrowing by rabbits exposes metalliferous spoil which would be available to other grazing animals, as well as re-exposing toxic material to wind blow (Figure 32).

(2) Long term viability of the vegetation on the main sites will depend on metal levels remaining non phytotoxic. Should the vegetative cover eventually fail, then long term stability would be affected, erosion would begin and spoil would be re-exposed.

Figure 32. Burrowing of Restored Spoil by Rabbits.

Although Dieback could be arrested by an inorganic fertiliser, it would be preferable to use a digested solid sewage sludge application to further provide the organic matrix, and to regulate the uptake of heavy metals through the root system.

(3) Benefits are to be accrued in terms of fertiliser and herbage growth from sheep grazing. A strict management regime should be exercised implementing short periods (2-3 weeks) of heavy grazing rather than continuous light grazing to deter accumulation internally of heavy metals. Long term health effects of this are unknown. Local experience and recent lamb research suggests no sheep under one year of age and no cattle should be grazed. Grazing should be implemented only in the summer months when herbage growth is adequate to prevent sheep ingesting spoil/sewage sludge containing heavy metals.

Periodic checks (every 2 years) on herbage metal levels, especially lead, should be made prior to grazing. If metal levels increase then fertiliser applications may be necessary. Herbage samples should be taken at the same time of year since variations will occur according to date of sampling and seasonal changes in the growth rate of vegetation. Sheep access may then require to be further regulated if metal levels in herbage increase significantly.

(4) The most suitable reclamation mix for direct seeding onto metalliferous waste outlined in this study is Trial Mix 1 with wood mulch. However in future work it would be beneficial to add a legume component for long term conversion of nitrogen from the atmosphere into a usable form in what is a nutrient deficient substrate.

(5) Any further work in this field should involve a comprehensive sampling of materials. Heterogeneity is a characteristic feature of metalliferous spoil and the physical and chemical characteristics of the waste vary greatly between areas and within an individual site depending on the parent rock and the stage of processing at which the spoil was discarded. These may affect suitability for plant growth and type as well as restoration. The factor of pH would clearly be very important, since it is likely that direct seeding of an acidic metalliferous waste would be unsuccessful and trial amendment covers would be needed.

Where amendment covers are necessary it is clear from the present work that further trials on calcareous and acidic wastes are required to determine satisfactory type and depth, but only if non-toxic herbage is required for grazing purposes. Options for the latter include the use of a relatively coarse/granular amendment of between 100mm and 150mm to deter movement of soluble metals by capillary action. Where adjacent materials differ in terms of structure an important barrier to capillary action is made. It is clear from the present trial plot exercise that even the colliery waste was too fine for this purpose and a more rough material would be required. It may be that an intermediate layer of coarse material made next to the spoil before addition of finer amendments would serve this purpose.

(6) Applications of organic materials (e.g. sewage sludge) should be made prior to seeding at between 150 and 200 tds ha^{-1} or split into three separate top dressings over a three year period.

(7) Further work should anticipate early drainage requirements and account for high volumes of silt run off.

4.5.4 Implications for The Local Community

Many studies (e.g. Charney et al, 1983) have demonstrated that reducing lead levels in house dust can significantly reduce blood lead levels in children in the short term. The important objective of the present lead abatement exercise is to guarantee a long term fall in air and dustfall lead concentrations and ultimately blood lead levels, in both the interior and exterior environments and communities of Leadhills and Wanlockhead.

Human blood lead levels in the area are undoubtedly high for a rural site, and although further blood lead concentrations have not been determined since spoil restoration it is likely from Moffat (1989) that health benefits should have been accrued. Moreover there has been a significant reduction in lamb deaths on grazing land which was formerly adjacent to the metalliferous waste.

5. CONCLUSIONS AND RECOMMENDATIONS

The purpose of this final section is to summarise the main findings from the human, lamb and spoil abatement studies and to highlight recomendations and important links between them.

5.1 THE HUMAN STUDY

Consideration of environmental factors in the former mining area indicated a general increase in residual lead exposure which has persisted since closure of the mines in the 1930s. In Leadhills and Wanlockhead lead concentrations in garden soil, in house dust, on hand wipes, on kitchen surface wipes and in airborne dust were elevated by around 30, 5, 3.5, 3 and 15 times, respectively, compared to the control village of Moniaive. Blood lead levels showed an excess of about 45% and 50% in men and women, respectively, and 70% in children in Leadhills/Wanlockhead compared to the control. The blood lead levels in the contaminated area were very similar to levels found in many British cities, but fall within EEC guidelines for populations. Recent research has however, suggested harmful effects particularly for children exhibiting blood lead levels similar to those found in the former mining villages. Measurement of lead in unwashed hair samples confirmed doubts on its usefulness as a measure of body lead status.

Of those environmental variables measured in both areas as possible determinants of blood lead, water lead which was generally low and within EEC recommendations, proved to be the most significant factor, explaining 11% of blood lead variability. Given the high lead concentrations in other environmental variables, this finding lends strong support to recent W.H.O. proposals for a five fold decrease in water lead concentrations

from 50 to 10 $\mu g l^{-1}$ to be followed. Hand lead accounted for 6%, airborne dust lead 3%, and kitchen surface and house dust lead less than 1% of variation, and were together less important as determinants of blood lead than water lead. However, these variables should not be neglected in seeking to reduce levels of contamination in Leadhills and Wanlockhead.

The data for hand lead indicated a large excess in the contaminated area particularly in men and children, and showed that there is a potential transfer of lead from hands entering the body. The 'dust-hand-mouth' transfer of lead is especially important for young children who may well also ingest soil or dust.
Mothers have been advised to train children and to ensure that hands are washed frequently - particularly before meals - and that mouthing of play-things or other objects is avoided.

Significant correlations in the data suggested that there was a transfer of lead from airborne dust and house dust to kitchen food preparation surfaces : kitchen wipe and blood lead correlations were also found.
Householders have been advised to implement frequent dusting, washing and vacuuming of all household surfaces, particularly in the contaminated area, in an attempt to lower blood lead concentrations through disruption of the house dust-hand-mouth lead pathway.

Garden soil lead and consumption of locally grown vegetables were not found to be associated with raised blood lead levels. Some vegetable concentrations exceeded government safety limits, but the situation was not sufficently serious to advise against the limited consumption of these.
Care in personal hygiene has however been advised in both the domestic preparation of vegetables which might be contaminated by soil/spoil particles and by those

involved in their cultivation (particularly men in the former mining area)..

A fifteen fold excess existed for lead measurements in external airborne dust in Leadhills and Wanlockhead compared with the control village, with dust lead from the spoil heaps probably contributing lead to all other environmental variables measured.
Study participants (particularly children) have been urged to play on 'clean' grassed areas outwith the home, and to avoid playing and motorbiking on spoil material. However, the long-term aim must be to tackle the sources of contamination of both the external and domestic environments.

5.2 THE LAMB STUDY

An examination of soil and herbage lead showed a sizeable increase of almost 50 fold and 17 fold, respectively, on the contaminated farm, compared with the control. Zinc and copper variables were also elevated in Wanlockhead indicating signs of common environmental contamination. Low macronutrient concentrations of calcium and phosphorus in soils and herbage were evident on both farms. The principal accumulation of lead in lamb blood occurred early in life with approximately five, six, five and four fold elevations evident in Wanlockhead lambs compared to those in Moniaive at 1, 4, 8 and 12 weeks, respectively. By twelve weeks of age, blood lead levels in Wanlockhead lambs were comparable to their dams, although ewes on the contaminated farm had blood lead concentrations more than twice those on the control farm. Plasma copper concentrations were significantly lower in ewes and twelve week old lambs grazing in Wanlockhead compared with the control, although there was no evidence of general hypocupraemia.

Organ tissue from lamb casualties in the two areas

indicated a six, fifteen and fifty fold lead excess in livers, bone and kidney, respectively, in Wanlockhead compared to Moniaive. Liver copper concentrations in Wanlockhead lambs were marginally subnormal.

The present results strengthened the evidence for an association between lead exposure and death rates in lambs in Wanlockhead, by relating clinical signs and blood lead concentrations. Blood lead levels at 1 and 4 weeks of age indicated a better predictive than diagnostic role in the development of the disorder by 8 and 12 weeks. Abnormally high plasma phosphorus concentrations in Wanlockhead lambs at 4 weeks of age may reflect diminished uptake of the mineral by the skeleton through high lead concentrations. Ewes showed no evidence of the locomotor disorder, and reproduced successfully on contaminated land.

Ewe milk lead concentrations were five times higher on the contaminated farm compared with the control, indicating mammary transfer of lead to be an important pathway of contamination for lambs. The ingestion of milk is particularly important, not only in terms of metal content, but because it may determine the amount of lead absorbed from other dietary sources such as soil / spoil, herbage and water. This probably explains the high gastrointestinal absorption of lead in the early weeks of the lamb's life when they are largely on a milk diet. As the grass intake of the diet increased, lead absorption decreased: an immature gut in the early weeks of the lamb's life probably further added to increased absorption of lead.

Supplementation of the lamb's diet with ascorbic acid did not prevent development of the locomotor disorder in the contaminated area. On the other hand, calcium and phosphorus supplements were particularly effective, with lowered blood lead increases, no clinical signs of the

disorder and good locomotor ability evident in treated lambs.

Farmers in the contaminated area have been advised to place 'Rumivite' blocks rich in calcium and phosphorus in areas where sheep and lambs tend to congregate; to restrict access of sheep, where possible, to heavily polluted land through fencing; and if feasible, to remove lambs from lead-polluted ground until they are at least eight weeks of age.

5.3 ASSOCIATIONS BETWEEN HUMAN AND LAMB STUDIES

The confirmation of a period of greatest vulnerability to lead toxicity in the first twelve weeks of the lamb's life raises the question of whether or not there is a similar period in childhood. Blood lead levels in lambs from the contaminated area were between 2.5 and 3.5 times higher than their dams over the first eight weeks of life. While children's blood lead levels were only 1.3 times higher than their mothers in Leadhills and Wanlockhead, no blood samples were taken from breast-fed or bottle-fed children. The differences between species may also reflect a number of other parameters. For example, children in the 'protected' internal environment of the home are subject to a lesser degree of lead exposure compared to lambs grazing exterior land. Nevertheless, current studies at Moredun Research Institute indicate that barely detectable increases in milk lead in ewes cause substantial increases in blood lead in the suckling lamb (N.F. Suttle, personal communication). Relationships between milk and blood lead should be studied in mothers and their breast-fed children.

Despite the lower exposure to lead it is possible that increased dietary intakes of macronutrients such as phosphorus and calcium have a similar protective effect

on children to that seen in lambs. This may also be an area worthy of future research, leading to increased parental and school awareness of the effects of nutrition on lead absorption. The need for protective measures would be increased if early exposure to lead in children had adverse effects on bone development even remotely related to those seen in lambs. The measurement of lead in blood will underestimate accumulation in the skeleton in all species. Although bone lead is metabolically inert in most circumstances it is likely that a release occurs during pregnancy, under conditions of stress and during infection in both species.

5.4 SPOIL RESTORATION

The long-term residual effects of the former lead-mining activity on human and livestock populations in Leadhills and Wanlockhead highlighted the need for measures to reduce contamination at source. A productive and sustainable grass sward has been established on small scale trial plots on spoil with amendment covers of local soil, domestic refuse and colliery spoil at depths of between 150 and 450mm. In addition, full-scale directly hydroseeded spoil trials have succeeded on the dry residue of former tailing lagoons.

Control trial plots seeded directly onto spoil failed in the second year without fertiliser application. Plots seeded with heather litter and seed failed to germinate. Heavy metal accumulation in trial plot herbage was high and would preclude grazing capability. However, following artificial fertiliser and sewage sludge applications onto hydroseeded spoil, both 'metal tolerant' and conventional reclamation seed mixes with peat and wood mulches have succeeded for at least seven years. Heavy metal accumulation was lower than in the trial plots allowing controlled grazing by sheep. The fact that sheep were largely able to graze the reclaimed spoil heaps without

reaching blood lead concentrations found on the contaminated farm, is eloquent testimony to the effectiveness and importance of establishing ground cover on the sources of contamination.

The restoration of a two hectare site in Leadhills and a similar six hectare site in Wanlockhead within each of the village areas has greatly reduced dispersal by wind and water erosion of the finest sand/silt spoil material in the area. Relatively coarse spoil remains on the periphery of both villages, largely preserved at present as Sites of Special Scientific Interest. It is clear however, that the material will be further broken down over time and that lead will become more available for ingestion and absorption by the local communities.

Landowners have been advised on restored spoil maintenance proposals, and to cease transportation of spoil for use as landfill, road 'bottoming' and path construction.

5.5 CONTINUING THE EFFORTS TO REDUCE LEAD CONTAMINATION

Clinical health effects of lead in the form of a locomotor disorder and/or death are much in evidence in lambs grazing in Leadhills and Wanlockhead. Whether asymptomatic lead concentrations found in children in these villages might be harmful remains a matter for deliberation. Child population numbers are too small (Table 1) to produce significant longitudinal evidence that lead may impair psychometric or cognitive function. A mean blood level of 17.6 +/- 5.4 in children is nonetheless at least as high or higher than recent studies claiming adverse effects on health. Risks with child health cannot be taken, and despite the problems, the situation is largely preventable. The above community education and strategies will increase awareness and hopefully result in a sustained effort to reduce exposure

to lead in the contaminated communities. The long term stabilisation of fine surface dust in the former tailing lagoons should reduce contamination in both man and animals substantially. The monitoring and the education process should clearly be a continuing process.

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7. APPENDICES

APPENDIX 1

Mean lamb blood concentrations of lead, copper, zinc, phosphorus, calcium and ascorbic acid on the two farms.

	AGE OF LAMB IN WEEKS							
	1		4		8		12	
	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)
LEAD (μgml^{-1})								
Wanlockhead	24	1.09(0.74)	23	1.45(0.89)	18	1.03(0.42)	12	0.67(0.31)
Moniaive	18	0.23(0.03)	18	0.23(0.04)	18	0.18(0.03)	18	0.15(0.04)
		***		***		***		***
COPPER (μgml^{-1})								
Wanlockhead	24	0.64(0.15)	23	0.65(0.21)	18	0.86(0.10)	12	0.69(0.24)
Moniaive	18	0.53(0.13)	18	0.74(0.33)	18	0.83(0.23)	18	0.91(0.12)
		*						**
ZINC (μgml^{-1})								
Wanlockhead	24	0.78(0.19)	23	0.82(0.17)	18	0.89(0.11)	12	0.79(0.14)
Moniaive	18	0.88(0.27)	18	0.69(0.08)	18	0.76(0.05)	18	0.91(0.10)
				**		***		*

Continued overleaf .....

APPENDIX 1 (contd.)

	AGE OF LAMB IN WEEKS							
	1		4		8		12	
	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)	n	Mean (sd)
PHOSPHORUS ($mmol\,l^{-1}$)								
Wanlockhead	24	2.54(0.51)	23	3.66(1.03)	18	2.70(0.25)	12	2.21(0.66)
Moniaive	18	2.76(0.31)	18	2.17(0.04)	18	3.03(0.34)	18	3.28(1.03)
				***		**		**
CALCIUM ($mmol\,l^{-1}$)								
Wanlockhead	24	2.75(0.29)	23	2.54(0.34)	18	2.84(0.25)	12	2.49(0.37)
Moniaive	18	3.07(0.11)	18	2.79(0.19)	18	2.77(0.08)	18	2.33(0.34)
		***		**				
ASCORBIC ACID ($\mu g\,ml^{-1}$)								
Wanlockhead	-	- -	-	- -	18	8.81(2.14)	-	- -
Moniaive	-	- -	-	- -	17	10.94(2.36)	-	- -
						**		

Note 1: Mean lead levels calculated using a two-sample T-test, weighted to allow for different variances. Analysis of variance used for all others.

2: $^{***}p<0.001$; $^{**}p<0.01$; and $^{*}p<0.05$.

APPENDIX 2

Effects of treating Wanlockhead lambs for two weeks with calcium and phosphorus (CA/P) or ascorbic acid (AA) supplements on blood lead, plasma AA and plasma iron concentrations. Analysis of variance used to test for significance in blood lead, plasma ascorbic acid and plasma iron, according to treatment.

	WEEK 0			WEEK 1			WEEK 2		
	n	Mean	(sd)	n	Mean	(sd)	n	Mean	(sd)
TREATMENT				LEAD (μgml^{-1})					
CA/P	8	1.32	(0.69)	8	1.50	(0.79)	8	1.44	(0.48)
AA	8	1.29	(0.91)	8	2.16	(0.84)	6	1.74	(0.23)
CONTROL	8	1.23	(0.27)	8	1.74	(0.37)	7	2.02	(0.62)
				ASCORBIC ACID (μgml^{-1})					
CA/P	8	6.02	(4.87-7.44)	8	6.24	(5.10-7.62)***	8	8.97	(7.31-10.99)**
AA	8	5.85	(4.12-8.29)*	8	19.16	(11.32-32.36)	6	20.35	(17.70-23.39)
CONTROL	8	8.48	(7.53-9.53)*	8	6.70	(5.41-8.30)	7	8.54	(5.98-11.67)
				IRON (μgml^{-1})					
CA/P	8	4.50	(0.61)	8	4.44	(0.98)	8	3.13	(0.82)
AA	7	3.99	(1.83)	8	4.78	(0.67)	6	4.39	(1.48)
CONTROL	8	4.77	(0.81)	8	4.53	(0.92)	7	3.91	(1.03)

Note 1: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.
2: Geometric means and 95% ranges for Ascorbic Acid obtained after log transformation.

APPENDIX 3

Accumulation of Heavy Metals in Herbage from Trial Plots.

LEAD

Depth (mm)	150	300	450
1986			
Local Soil	115,49	90,122	106,82
Domestic Refuse	64,50	68,104	86,72
Colliery Spoil	66,36	53,55	-
1987			
Local Soil	38,58	68,75	49,60
Domestic Refuse	29,41	53,29	42,37
Colliery Spoil	61,15	52,42	-
1988			
Local Soil	21,73	48,35	21,48
Domestic Refuse	21,35	22,23	35,11
Colliery Spoil	46,11	28,12	-
1989			
Local Soil	66,105	105,80	48,153
Domestic Refuse	30,40	63,65	95,106
Colliery Spoil	11,102	10,103	-

continued overleaf.....

APPENDIX 3 Continued

ZINC

Depth (mm)	150	300	450
1986			
Local Soil	55,65	75,45	46,76
Domestic Refuse	70,38	62,82	74,60
Colliery Spoil	38,50	43,43	-
1987			
Local Soil	53,22	24,28	67,37
Domestic Refuse	53,35	32,38	34,55
Colliery Spoil	23,25	23,25	-
1988			
Local Soil	26,27	27,25	16,28
Domestic Refuse	35,26	26,47	17,26
Colliery Spoil	25,20	22,23	-
1989			
Local Soil	29,27	62,27	29,22
Domestic Refuse	38,29	42,54	33,55
Colliery Spoil	19,22	11,25	-

continued overleaf.....

APPENDIX 3 Continued

COPPER

Depth (mm)	150	300	450
1986			
Local Soil	6.8,7.2	6.2,5.6	6.3,5.7
Domestic Refuse	6.6,7.0	7.0,7.4	5.7,5.9
Colliery Spoil	6.5,6.0	6.1,5.8	-
1987			
Local Soil	6.6,6.4	5.4,6.2	6.0,6.8
Domestic Refuse	7.0,5.7	7.2,6.8	5.9,6.1
Colliery Spoil	5.9,6.0	5.7,6.9	-
1988			
Local Soil	6.6,7.2	6.8,5.4	6.8,4.6
Domestic Refuse	7.1,6.7	6.7,7.2	5.5,6.7
Colliery Spoil	5.6,6.5	5.9,5.8	-
1989			
Local Soil	4.6,5.3	6.8,5.3	5.3,3.4
Domestic Refuse	4.7,4.8	5.2,4.5	5.5,5.7
Colliery Spoil	3.2,4.0	4.0,4.8	-

continued overleaf.....

APPENDIX 3 Continued

CADMIUM

Depth (mm)	150	300	450
1986			
Local Soil	0.10,0.18	0.08,0.12	0.07,0.07
Domestic Refuse	0.07,0.11	0.07,0.05	0.08,0.10
Colliery Spoil	0.07,0.05	0.01,0.03	-
1987			
Local Soil	0.09,0.10	0.15,0.05	0.08,0.13
Domestic Refuse	0.12,0.15	0.04,0.03	0.05,0.05
Colliery Spoil	0.15,0.18	0.05,0.04	-
1988			
Local Soil	0.14,0.20	0.08,0.04	0.16,0.08
Domestic Refuse	0.05,0.03	0.04,0.04	0.02,0.04
Colliery Spoil	0.06,0.07	0.05,0.03	-
1989			
Local Soil	0.18,0.22	0.20,0.21	0.12,0.18
Domestic Refuse	0.11,0.12	0.09,0.16	0.16,0.12
Colliery Spoil	0.09,0.14	0.07,0.11	-

Note 1: Individual results are for each replicate trial plot situation, except those seeded directly onto spoil (n=2).

2: Heavy metals ($\mu g g^{-1}$).

APPENDIX 4

Mean accumulation of Heavy Metals and Macronutrients in Herbage from Hydroseeding Trials.

	Pb	Zn	Cu	Cd	N	P	K
1987							
Trial 1	14.6	38	6.0	0.23	1.80	0.24	1.67
	(12.0-20.0)	(24-54)	(5.5-6.4)	(0.18-0.30)	(1.40-2.18)	(0.18-0.31)	(1.50-1.75)
Trial 2	16.0	41	6.4	0.18	1.89	0.22	1.85
	(12.0-22.0)	(25-61)	(5.8-7.2)	(0.14-0.22)	(1.54-2.25)	(0.20-0.24)	(1.39-2.10)
Trial 3	29.3 **	82 **	5.9	0.20	1.92	0.20	1.80
	(26.0-34.0)	(49-102)	(5.6-6.2)	(0.10-0.30)	(1.61-2.30)	(0.16-0.26)	(1.78-1.84)
1988							
Trial 1	10.8	45	6.2	0.19	1.88	0.23	1.87
	(5.0-19.0)	(30-68)	(5.1-6.9)	(0.10-0.31)	(1.53-2.31)	(0.20-0.27)	(1.81-1.95)
Trial 2	9.0	36	5.3	0.15	1.90	0.21	1.94
	(6.0-12.0)	(27-42)	(4.1-7.3)	(0.11-0.25)	(1.36-2.22)	(0.17-0.23)	(1.68-2.10)
Trial 3	11.3	35	6.3	0.25	1.97	0.21	1.83
	(5.0-21.0)	(23-44)	(5.6-7.2)	(0.08-0.48)	(1.64-2.15)	(0.18-0.23)	(1.63-2.06)

Continued overleaf .....

APPENDIX 4 (Contd.)

	Pb	Zn	Cu	Cd	N	P	K
1989							
Trial 1	13.2 (10.0-20.0)	34 (18-42)	6.8 (5.4-8.6)	0.28 (0.15-0.42)	2.12 (1.54-2.70)	0.26 (0.24-0.28)	1.86 (1.74-2.01)
Trial 2	13.0 (10.0-16.0)	34 (24-48)	6.9 (5.4-8.7)	0.35 (0.25-0.48)	2.25 (1.54-3.02)	0.28 (0.23-0.30)	2.00 (1.75-2.18)
Trial 3	22.2 ** (12.0-33.0)	64 ** (14-115)	7.5 (5.5-8.8)	0.78 * (0.23-1.40)	2.22 (1.61-2.78)	0.26 (0.18-0.26)	1.87 (1.76-1.95)
1990							
Trial 1	13.0 (8.0-19.0)	57 (37-78)	6.0 (4.3-7.4)	0.38 (0.22-0.54)	1.55 (1.40-1.74)	0.28 (0.26-0.29)	1.60 (1.40-1.81)
Trial 2	13.0 (9.0-16.0)	40 (30-52)	5.7 (4.1-7.1)	0.25 (0.14-0.34)	1.30 (1.02-1.62)	0.26 (0.22-0.30)	1.40 (1.24-1.54)
Trial 3	14.0 (8.0-17.0)	54 (44-73)	6.0 (5.2-7.4)	0.40 (0.32-0.55)	1.42 (1.34-1.49)	0.23 (0.19-0.25)	1.32 (1.17-1.46)

Note 1: Heavy metals ($\mu g g^{-1}$); Macronutrients (%).
2: All values are means (ranges) of four samples taken from each trial seed area.
3: Analysis of variance used to test for significance in trials where $^{*}p<0.05$ and $^{**}p<0.01$.

APPENDIX 5

Grazing Sheep Blood Trials, 1989, 1990.

	Pb 1989		Pb 1990	
	RESTORED	CONTROL	RESTORED	CONTROL
Time (weeks)	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)
0	0.17 (0.10-0.28)	0.19 (0.11-0.24)	0.28 (0.24-0.36)	0.28 (0.23-0.33)
1	0.28 (0.17-0.35)	0.21 * (0.16-0.26)	- -	- -
2	- -	- -	0.34 (0.29-0.45)	0.24 *** (0.21-0.27)
3	0.39 (0.32-0.49)	0.23 *** (0.18-0.29)	- -	- -
5	0.38 (0.24-0.45)	0.23 *** (0.16-0.29)	0.34 (0.26-0.41)	0.24 *** (0.20-0.28)
7	0.31 (0.21-0.42)	0.24 ** (0.17-0.29)	- -	- -
8	- -	- -	0.58 (0.51-0.66)	0.50 ** (0.44-0.54)
9	0.40 (0.35-0.48)	0.32 ** (0.21-0.37)	- -	- -
10	- -	- -	0.40 (0.33-0.47)	0.27 *** (0.24-0.30)
11	0.37 (0.28-0.42)	0.26 *** (0.19-0.37)	- -	- -
13	- -	- -	0.36 (0.29-0.42)	0.27 *** (0.23-0.34)
16	- -	- -	0.39 (0.31-0.52)	0.29 *** (0.26-0.31)
19	- -	- -	0.48 (0.42-0.57)	0.37 *** (0.31-0.46)

Note 1: Pb in $\mu g ml^{-1}$.
2: n = 10 for each area.
3: Analysis of variance used to test for significance, where $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

APPENDIX 5 (Contd.)

Time (weeks)	Zn 1989 RESTORED Mean (Range)	Zn 1989 CONTROL Mean (Range)	Zn 1990 RESTORED Mean (Range)	Zn 1990 CONTROL Mean (Range)
0	0.88 (0.78-1.05)	0.97 (0.82-1.09)	0.75 (0.54-1.06)	0.76 (0.54-1.06)
1	1.01 (0.86-1.16)	1.01 (0.90-1.14)	- -	- -
2	- -	- -	0.90 (0.78-1.05)	0.84 (0.67-1.01)
3	1.00 (0.92-1.13)	0.99 (0.88-1.11)	- -	- -
5	1.12 (0.99-1.29)	1.08 (0.95-1.23)	0.92 (0.69-1.19)	0.87 (0.62-1.20)
7	0.99 (0.84-1.16)	0.91 (0.72-1.09)	- -	- -
8	- -	- -	0.84 (0.46-1.01)	0.79 (0.52-1.11)
9	1.03 (0.86-1.17)	0.83 ** (0.62-1.14)	- -	- -
10	- -	- -	1.24 (0.99-1.36)	1.03 ** (0.92-1.25)
11	0.99 (0.89-1.14)	0.92 (0.51-1.09)	- -	- -
13	- -	- -	0.89 (0.74-0.97)	0.80 (0.61-1.05)
16	- -	- -	1.06 (0.95-1.23)	0.95 ** (0.79-1.03)
19	- -	- -	0.88 (0.78-0.96)	0.80 * (0.67-0.92)

Note 1: Zn in $\mu g ml^{-1}$.
2: n = 10 for each area.
3: Analysis of variance used to test for significance, where $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

APPENDIX 5 (Contd.)

Time (weeks)	Cu 1989 RESTORED Mean (Range)	Cu 1989 CONTROL Mean (Range)	Cu 1990 RESTORED Mean (Range)	Cu 1990 CONTROL Mean (Range)
0	0.88 (0.62-1.13)	0.96 (0.88-1.12)	0.75 (0.53-0.92)	0.76 (0.59-0.90)
1	0.84 (0.58-1.01)	0.96 * (0.83-1.14)	-	-
2	- -	- -	0.86 (0.66-1.15)	0.85 (0.62-0.97)
3	0.87 (0.64-1.00)	0.92 (0.72-1.12)	- -	- -
5	0.82 (0.68-0.93)	0.85 (0.69-0.97)	0.84 (0.68-1.07)	0.87 (0.62-0.97)
7	0.82 (0.54-0.96)	0.95 * (0.77-1.14)	- -	- -
8	- -	- -	0.76 (0.36-0.91)	0.86 (0.67-1.34)
9	0.80 (0.65-0.90)	1.04 ** (0.83-1.56)	- -	- -
10	- -	- -	0.83 (0.46-1.24)	0.95 (0.53-1.52)
11	0.74 (0.56-0.89)	0.99 * (0.74-1.54)	- -	- -
13	- -	- -	0.81 (0.43-1.06)	0.88 (0.49-1.12)
16	- -	- -	0.86 (0.40-1.25)	0.81 (0.40-0.93)
19	- -	- -	0.82 (0.42-1.07)	0.83 (0.46-1.12)

Note 1: Cu in $\mu g ml^{-1}$.
2: n = 10 for each area.
3: Analysis of variance used to test for significance, where $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

APPENDIX 6

Publications arising from work in this thesis.

Blood lead determinants of a population living in a former lead mining area in Southern Scotland

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Abstract

The impact of high environmental lead levels on public health is currently under much debate. Such a situation exists in two former lead mining villages set in the Southern Uplands of Scotland, where the environment is heavily contaminated through past mining activity. A survey was conducted based on representative samples of male and female adults and of all children living in the area, to examine the distribution of blood lead levels and to compare this with the distribution in residents in a control area. Possible routes of exposure including the determination of lead in domestic water, in house dust, in airborne dust, on food preparation surfaces, on hands, in garden soils and through home grown vegetable consumption were investigated. The results indicate that there is a general increase in lead exposure in environmental variables in the contaminated area, while blood lead levels show an excess of between 45 and 70 percent compared with the control. The determinants of blood lead are discussed through correlation and multiple regression analysis.

Introduction

The involuntary elevation of human lead levels from lead contaminated environments caused by past and present industrial activity has been widely noted in numerous studies (Barltrop *et al.*, 1975; Gallacher *et al.*, 1984; Yankel *et al.*, 1977).

In view of this previous research work carried out in other lead contaminated areas, it seemed reasonable to suggest that similar circumstances might prevail in this particular study area. It is known from previous work (Moffat, 1982) that young animals are adversely affected by high environmental lead levels and some public disquiet has been noted in terms of health effects for the local population.

The investigation took place in the former lead mining villages of Leadhills (O.S. Grid Coordinate 885 150) and Wanlockhead (O.S. Grid Coordinate 875 130) situated in Southern Scotland. The two villages have a combined population of 400.

The main rocks in the area belong to the Silurian and Ordovician periods and are formed of strongly folded shale and greywackes. Mining operations date back to Roman times and at their peak in the late eighteenth, and early nineteenth century, produced one- tenth of the total UK lead ore. Extraction ceased in the 1930s but the legacy of past industry is evident in today's landscape. Most notable perhaps are the extensive spoil heaps containing high, though variable amounts of toxic metals. Local soils contain considerable amounts of lead and zinc and it is estimated that overall 4,000 acres of land are subject to at least some contamination (Moffat, 1982).

To serve as a comparison, a control village, Moniaive with a population of 400, was surveyed 26 miles south west of the study area (O.S. Grid Coordinate NX 780 910), which was similar in all possible respects other than having obvious lead pollution. This village lies in the same general belt of Silurian strata as Leadhills and Wanlockhead.

Methods

(i) *Sampling*

A random sample of one third of the adult population was drawn from the electoral register in Leadhills and Wanlockhead, with a random one-sixth selected in Moniaive. Young persons aged between 12 and 17 years were included from both areas giving an overall "adult" total of 130 and 87 respectively. In addition all those children aged under 12 were sampled numbering 31 in Leadhills and Wanlockhead, and 19 in Moniaive.

Environmental samples were collected in February 1984 and again in June 1984 to check on reproducibility of results with respect to season. Venous blood samples were taken twice from all the adults, but from young children aged under 12 only once (June 1984).

Soil samples were collected from a random one third of the gardens (where possible from the rear of the house) using a stainless steel trowel to a depth of 15 cms.

House dust samples were collected from the vacuum cleaner bag of all those households participating in the study.

Lead on hands and kitchen food preparation surfaces was determined using a wet wipe technique (Gallacher *et al.*, 1984; Sayre *et al.*, 1974) while first flush and random daytime samples of water were taken from the cold tap of every household.

Air borne dust lead was monitored in a random 31 gardens in Leadhills and Wanlockhead, with a further 10

Table 1 *Mean blood lead levels (µmol/l) in the two areas.*

	Men		*Women*		*Children*	
	n	*Mean* (*sd*)	*n*	*Mean* (*sd*)	*n*	*Mean* (*sd*)
Leadhills + Wanlockhead	55	0.77 (0.26)	71	0.60 (0.25)	22	0.85 (0.26)
Moniaive	43	0.53 (0.22)	41	0.40 (0.15)	15	0.50 (0.16)
		***		***		***

Conversion: SI to traditional units -
lead 1 µmol/l = 20.7 µg/100 ml.
Student's t-test used to test for significance where *** = $p<0.001$).

in Moniaive, using the moss bag technique pioneered by Goodman and Roberts (1975). Initially set up in March 1984, the moss bags were renewed usually every 30 days or so until December 1984, when monitoring ceased.

Finally, a record was constructed for each individual as to the proportion of vegetables eaten which had been grown at home. This information was categorised according to the following: (1) all or most (2) some (3) none.

(ii) *Laboratory analysis*

Hand wipes, kitchen wipes, garden soils, and moss bags were analysed using flame atomic absorption spectro-photometry. Dust samples were determined by flameless atomic absorption. The air- dried soil was ground to pass through a 2-mm mesh sieve and lead extracted using an aqua regia digestion. Dust samples were dried, lightly ground and sieved through a 2-mm mesh and lead extracted using hot concentrated nitric acid (Davies *et al.*, 1985). The other samples were oven dried at 100 °C and digested in concentrated nitric acid.

Determination of lead in water followed the method outlined by the DOE (1980) while blood was analysed by the method of Stoeppler, Brandt and Rains (1978).

Where possible, 1 in 10 samples were run as blind duplicates as a check on laboratory estimations. The coefficient of variations (Sd/mean x 100 percent) of blood lead estimation based on 75 blind duplicates of venous blood was 5 percent; 9 percent for garden soil (6 duplicate dwellings); 11 percent for garden vegetables (15 duplicate samples); 22 percent for house dust samples (57 duplicate samples); and 18 percent for air lead (9 duplicates run on two separate occasions). These values indicate that the precision in reproducibility of estimations is within acceptable limits.

In order that parametric statistical tests could be applied, all data with the exception of blood (normally distributed) and water (cube root transformation) were transformed to their common logarithms.

Results

Blood lead

The mean results from February and June for Leadhills and Wanlockhead, and the control village Moniaive are shown in Table 1. In the former lead mining villages the mean value for men is 0.77 µmol/l Pb, for women 0.60 µmol/l and for children 0.85 µmol/l (Response rates of 96, 97 and 71 percent respectively). In Moniaive the results for blood lead are 0.53 µmol/l, 0.40 µmol/l and 0.50 µmol/l, for the corresponding categories (Response rates of 94, 100 and 79 percent respectively).

Environmental sources of lead

Comparisons of the distributions of the various environmental variables shown in Tables 2 and 3 indicate that there is a significant general increase in lead exposure in the contaminated area.

Perhaps the most striking contrast is in soil lead levels in the two areas with a geometric mean level of 6,902 µg/g in Leadhills and Wanlockhead, and 213 µg/g in Moniaive.

House dust samples have a geometric mean of 1,570 µg/g Pb in the contaminated area, and 320 µg/g Pb in the control village.

Geometric mean concentrations for hand lead are 28.5 µg/g, 12.2µg/g and 40.8 µg/g (for men, women and children respectively) in the contaminated area. In the control area, values are 8.1 µg/g, 4.7 µg/g and 10.5 µg/g. The geometric mean concentration of lead on kitchen surfaces in the old lead mining area is 14.1 µg/g and in Moniaive, 5.4µg/g.

Lead levels found in water in both areas are generally low, with a mean of 0.016 mg/l in Leadhills and Wanlockhead, and 0.011 mg/l in Moniaive. Results are for random daytime samples, as these are thought to be more representative of a person's intake than first flush water (DOE., 1977; Moore *et.al.*, 1977).

A geometric mean exposure to airborne dust lead of 2.19 µg/g/day was obtained in the contaminated area, and 0.15 in the control.

Table 4 summarises the data for blood lead levels

Table 2 *Environmental lead levels in the two areas. Garden soil house dust, kitchen surface, water and airborne dust lead from each dwelling.*

	Leadhills + *Wanlockhead*	Moniaive
A *Soil Lead* (µg/g)		
Number of samples	28	20
Mean	6902	213***
95% range	1,954 - 24,378	51 - 887
B *House dust lead* (µg/g)		
Number of samples	88	65
Mean	1570	320***
95% range	370 - 6,668	53 - 1,919
C *Kitchen surface lead* (µg/g)		
Number of samples	89	64
Mean	14.1	5.4***
95% range	1.5 - 130.6	1.2 - 25.0
D *Water Lead* (mg/l)		
Number of samples	89	66
Mean	0.016	0.011**
95% range	0.001 - 0.07	0.002 - 0.030
E *Airborne dust lead* (µg/g/day of exposure)		
Number of samples	30	10
Mean	2.19	0.15***
95% range	0.54 - 8.93	0.06 - 0.40

For A, B, C and E, the means, *etc.*, were obtained after log transformation. For D, cube root transformation was used (Sherlock *et al.*, 1982).
*** = $p<0.001$: ** = $p<0.01$
Hand wipe lead levels are shown separately in Table 3.

Table 3 *Hand wipe lead (µg/g) concentrations in the two areas.**

	Men		*Women*		*Children*	
	n	*Mean*	*n*	*Mean*	*n*	*Mean*
Leadhills + Wanlockhead	54	28.5	71	12.2	19	40.8
(95% range)	(1.5 - 543.3)		(1.5 - 99.8)		(8.6 - 192.7)	
Moniaive	41	8.1	40	4.7	14	10.5
(95% range)	(0.9 - 73.3)***		(1.3 - 17.0)***		(5.0 - 22.0)***	

Student's t-test used to test significance, where *** = $p<0.001$)
* Log transformation used.

according to the proportion of home-grown vegetables consumed by the subjects in the two areas. There is no evidence from these results of a stepwise increase in blood lead in relation to a greater consumption of home-grown produce.

Discussion

Determinants of blood lead levels

In order to investigate the relationships between blood lead levels and the independent environmental variables measured, simple correlation coefficients were calculated (Table 5).

Several authors have shown a positive correlation between blood lead and soil lead (for example, Yankel *et al.*, 1977). However, the present study is commensurate with a study in Derbyshire with no significant correlation observed between the two, indicating that lead in soil itself is a relatively minor source for exposure (Barltrop *et al.*, 1975).

House dust levels of lead as well as soil have been the subject of investigation for some time concerning the relationship of exposure and the amount of lead absorbed by humans (Landrigan *et al.*, 1975; Sayre *et al.*, 1974). The data shown in Table 5 clearly indicate there is no significant direct correlation between house dust lead and blood lead. For men, house dust lead contributes only 1 percent ($100r^2$) to blood lead and for women and children the figure is less that 1 percent in the contaminated area. Although clearly linked, correlation coefficients displayed in Table 6 for the environmental factors would imply that only about 8 percent of the variability in house dust lead can be explained by soil lead in Leadhills and Wanlockhead.

Hand wipe levels of lead reflect a large excess in the contaminated area suggesting that this route might well be important in explaining the elevated blood levels in Leadhills and Wanlockhead. There is a clear indication that for the adult group, men are more likely to be susceptible to this route of transfer of lead than women which is a probable reflection of differences in day to day activities. This is verified by correlation of hand and blood lead where a positive correlation was detected in only the male groups.

It is perhaps worth mentioning in this discussion of hand lead that there are some basic differences between adults and children in lifestyle and susceptibility to lead exposure which need further explanation. This stems from the knowledge that young children represent the highest risk group and tend to show higher blood lead levels than adults (Barltrop *et al.*, 1975; Gallacher *et al.*, 1984). It is evident that this study is no exception.

Young children exhibit the characteristic known as *pica* which can be defined as the ingestion of non-food matter either through direct ingestion of dirt and soil or dust, or through the mouthing of objects or hands contaminated by these. Hand wipes show a marked lead increase in children from Leadhills and Wanlockhead, and in Moniaive compared with the adult groups, and a significant difference between the contaminated and control area ($p<0.001$).

Several authors have demonstrated the feasibility of house dust as a source of lead for children, via the dust-hand-mouth route (for example. Sayre *et al.*, 1974). The present results for correlation analysis of house dust and hand lead overall shed further light on this subject. In Moniaive, significant positive correlations were established between the two factors of 0.35 ($p<0.05$) in men and 0.44

Table 4 *Blood lead levels (μmol/L) in the two areas in subjects grouped by their consumption of homegrown vegetables.*

	Homegrown vegetable consumption					
	None		*Some*		*All/Most*	
	n	*Mean (sd)*	*n*	*Mean (sd)*	*n*	*Mean (sd)*
Leadhills + Wanlockhead						
Men	23	0.72 (0.23)	24	0.71 (0.17)	8	1.07 (0.38)
Women	35	0.60 (0.25)	26	0.57 (0.27)	10	0.65 (0.23)
Children	10	0.81 (0.24)	10	0.91 (0.30)	2	0.71 (0.01)
Moniaive						
Men	10	0.58 (0.17)	20	0.43 (0.18)	13	0.63 (0.26)
Women	8	0.48 (0.20)	23	0.34 (0.10)	10	0.48 (0.14)
Children	5	0.39 (0.12)	9	0.53 (0.15)	1	0.77 (-)

Table 5 *Correlation coefficients for blood lead with the various environmental factors in the two areas.*

	Garden soil (n)	*House dust (n)*	*Hand wipe (n)*	*Kitchen surface (n)*	*Airborne dust (n)*	*Water (n)*
Leadhills + Wanlockhead						
Men	0.02 (15)	0.11 (53)	0.28* (54)	0.34* (54)	0.20 (19)	0.34* (53)
Women	-0.34 (27)	0.06 (70)	-0.11 (71)	-0.09 (70)	-0.02 (29)	0.43*** (70)
Children	-0.31 (7)	-0.02 (21)	0.38 (19)	-0.01 (21)	0.55 (9)	-0.14 (20)
Moniaive						
Men	0.43 (20)	0.30 (42)	0.41** (41)	0.31** (41)	0.65 (9)	0.24 (42)
Women	0.45 (10)	0.33* (40)	0.15 (40)	0.35* (40)	0.40 (5)	0.04 (40)
Children	0.22 (5)	-0.20 (14)	0.24 (14)	0.39 (14)	- (1)	0.52* (15)

* = $p<0.05$; ** = $p<0.01$ and *** = $p<0.001$

($p<0.01$) in women, and also in women from Leadhills and Wanlockhead ($r=0.33$, $p<0.01$), but not in men. Significant correlations were absent for both child groups, which is perhaps not unusual when the small sample size is taken into consideration, although if a relationship is evident in adults, then there is likely to be a similar, if not stronger route in children, according to individual behaviour. Interestingly, correlation analysis of garden soil and hand lead gave no significant positive relationship in either area or group (on average, $r=0.09$).

Although no direct correlation was observed for every age group in the two areas between blood lead and kitchen surface lead the environmental factors "house dust" and "airborne dust" both show significant positive correlations with kitchen surface lead. These results suggest that a pathway exists for lead passing into the food chain through contamination of kitchen surfaces (Table 6).

Overall, the data for the wet wipes intimate that in the area of high environmental lead, a reduction in blood lead concentration could well arise from improved standards of personal and domestic hygiene. The wet wipe results are in accordance with those found in an old lead mining area of Wales (Gallacher *et al.*, 1984).

Results for water lead (Table 2) and their subsequent

Table 6 *Correlation coefficients for the various environmental factors in the two areas.*

		Garden soil	*House dust*	*Kitchen surface*	*Airborne dust*	*Water*
Garden	L/W	1.00	0.29	0.32	0.30	- 0.32
soil	M	1.00	0.23	0.31	0.29	- 0.07
House	L/W		1.00	0.27**	0.20	0.05
dust	M		1.00	0.37**	0.38*	- 0.10
Kitchen	L/W			1.00	0.44*	0.06
surface	M			1.00	0.72*	-0.09
Airborne	L/W				1.00	0.00
dust	M				1.00	-0.01
Water	L/W					1.00
	M					1.00

* = $p<0.05$; ** = $p<0.01$
L/W = Leadhills/Wanlockhead; M = Moniaive

Table 7 *Multiple regression results on blood lead in the two areas*

Order of inclusion	*Accumulated percentage of variance explained*
Area	13.7
Group	23.4
Water	34.4
Hand	39.9
Airborne dust	43.0
Kitchen Surface	43.4
House dust	43.6

Regression equation

Blood Pb = 1.52 (water Pb)$^{-3}$ + 0.15 log (hand Pb)
+ 0.18 log (airborne dust Pb)
+ 0.06 log (kitchen surface Pb)
+ 0.06 log (house dust Pb) + constant*

*Constant values**

	Leadhills Wanlockhead	*Moniaive*
Men	-0.14	-0.05
Women	-0.25	-0.16
Children	0.01	0.10

Dependent variable: Blood lead
Independent variable: Area, group, house dust lead, hand lead, kitchen surface lead, airborne dust lead and water lead.
All data for the environmental levels (hand, airborne dust, kitchen surface, and house dust lead) transformed to logarithms. Water lead transformed using the cube root.

correlation with blood lead (Table 5) would indicate that there is a highly significant correlation for women and a significant correlation for men between the two factors in the contaminated area. In this connection, research by Elwood *et al.*, (1984) showed that water lead can be of significance, even at very low concentrations.

Airborne dust lead as measured by the moss bag technique showed no significant correlation with blood lead in Leadhills and Wanlockhead. However, the results give no indication of particle size of lead in air and cannot be transposed to represent air results as measured by standard air monitoring equipment. Nevertheless, the values for airborne dust lead are similar to other studies carried out in lead contaminated areas using similar techniques (Davies and White, 1981; Goodman *et al.*, 1975).

One of the most likely routes for soil lead to blood lead could be through the consumption of home grown produce in the contaminated villages. This could either be through direct ingestion or through handling contaminated soil and vegetables. The data in Table 4 display the relevant information. A significant difference was observed for male subjects grouped by their consumption of home grown vegetables compared with the control group ($p<0.001$). No such difference was evident for women. Furthermore, no stepwise increase is present for men, women, or children in blood lead with an increased consumption of vegetables indicating that there is no evidence of increased vegetable consumption contributing to a significant increase in blood lead.

Since the data overall suggest a multifactorial contribution of lead to blood, a stepwise multiple regression analysis was undertaken in an attempt to isolate the dependence of blood lead on the various environmental factors in the two areas. These results are presented in Table 7 along with the regression equation which can be used to predict the mean blood levels of a subject population.

Differences between the two areas in terms of "Area" and "Group" explain 13.7 percent and 9.7 percent respectively of the variance in blood lead levels. With these two variables entered into the model and controlled for, the respective contribution of each independent environmental variable to blood lead was then determined.

This exercise verified the importance of water lead as a contributor to blood lead, and by "explaining" 11 percent of the variance in blood lead showed that despite the low lead levels, water is the single most important factor in predicting blood lead levels. No other independent variable was of such significance, supporting work by Elwood *et al.* (1984) which points to the significance of relatively low water lead levels as a source of blood lead. Hand lead was relatively important explaining around 6 percent, airborne lead dust contributed 3 percent, while kitchen surface lead and house dust lead explained less than 1 percent of the variance.

Due to the small number of samples, garden soil results were not included in the regression analysis. However, from the results for correlations between soil and blood, it is unlikely that soil lead would make a further

contribution to explaining variance in blood lead.

A further examination of the data in Leadhills and Wanlockhead alone (n=55) generated the following regression:

$$\text{Blood Pb} = 1.59\ (\text{water Pb})^{-3} + 0.24\ \log\ (\text{hand Pb}) + 0.18\ \log\ (\text{airborne dust Pb}) - 0.03\ \log\ (\text{kitchen surface Pb}) - 0.03$$

Through this, water lead was again shown to be the best predictor, explaining 12.9 percent; hand lead in conjunction with water lead explained a further 13.6 percent; airborne dust lead 3.4 percent; and kitchen surface lead 0.2 percent. House dust lead no longer contributed, with the model "explaining" 29 percent of the variability in blood lead overall.

Seasonal variation

Blood lead levels in adults show a statistically significant difference between February and June (p<0.05 for men and p<0.001 for women). Similar studies of blood lead levels have also identified seasonal variability in blood levels (Stark *et al.*, 1982; WHO, 1977). Hand lead levels also exhibit seasonal variability. This phenomenon is probably best explained by saying that the main source of lead is likely to be the exterior environment. People tend to spend more time out of doors in the summer months involved in such occupations as gardening, which could feasibly contribute lead to both variables.

The reproducibility of results for kitchen surface lead, house dust lead and water lead remained constant over the sampling period.

Conclusions

Consideration of environmental factors in the former mining area would indicate that despite great variability there is a general increase in lead exposure, compared with the control. Moreover, blood lead levels show an excess of about 45-70 percent in Leadhills and Wanlockhead compared with Moniaive.

However, blood lead levels in the contaminated area are very similar, and in some cases lower than those found in some British cities and fall within EEC guidelines (DOE, 1981, 1982; Official Journal of the European Communities, 1980).

Of those environmental variables measured as possible determinants of blood lead, water lead which was generally low and within EEC recommendations, proved to be the most significant single factor in explaining variance in blood lead.

The data for hand wipes indicate a potential transfer of lead from hands entering the food chain. This is especially applicable to young children in Leadhills and Wanlockhead, whose hands should be washed frequently and prevented from mouthing objects which may be contaminated.

Significant correlations in the data suggest a transfer of lead from airborne dust and house dust to kitchen food preparation surfaces. This leads to advice for the householder in terms of domestic hygiene.

There is no evidence to suggest that the consumption of locally grown vegetables is associated with raised blood lead levels. Care in washing should however be exercised in the preparation of vegetables which might be contaminated by soil particles.

Finally, it is thought highly unlikely that there are any detectable effects for the residents of Leadhills and Wanlockhead. Blood lead levels show a relatively small elevation, therefore health effects from exposure to high environmental lead levels are improbable.

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